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Hormone therapy in elderly women; a comparative study with alendronate of the effects on bone, cardiovascular risk factors, periodontal conditions and quality of life

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Academic Dissertation

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Abbreviations

ALP	alkaline phosphatase
ANOVA	analysis of variance
AUC	area under the curve
BMD	bone mineral density
BMI	body mass index
BMS	burning mouth syndrome
CEE	conjugated equine estrogen
CHD	coronary heart disease
CI	confidence interval
CRP	C-reactive protein
CV	coefficient of variation
CTX	C-terminal cross-linked telopeptide of type I collagen
CVD	cardiovascular diseases
DMFT	diseased, missing, filled teeth (index)
Dpy	deoxypyridinoline
DT	diseased teeth
DXA	dual-energy X-ray absorptiometry
ERA	Estrogen Replacement in Atherosclerosis trial
ER	estrogen receptor
ERT	estrogen replacement therapy
ET-1	endothelin-1
E2	estradiol
E2V	estradiol valerate
FT	filled teeth
GCF	gingival crevicular fluid
GLA	γ-carboxyglutamic acid
HDL	high density lipoprotein
HERS	The heart and estrogen/progestin replacement study
HR	hazard ratio
HRQoL	health-related quality of life
HT	hormone therapy
ICTP	cross-linked carboxyterminal telopeptide of type I collagen
LDL	low density lipoprotein
Lp(a)	lipoprotein a
MMP	matrix metalloproteinase
MORE	Multiple Outcomes of Raloxifene Evaluation trial
MPA	medroxyprogesterone acetate
MWS	Million Women Study
NETA	norethisterone acetate
NNT	numbers needed to treat
NO	nitric oxide
NTX	aminoterminal cross-linked telopeptide of type I collagen
OC	osteocalcin
25(OH)D	25-hydroxyvitamin D
OPTG	panoramic tomography of the jaws
PEPI	Postmenopausal Estrogen / Progestin Interventions

PICP	carboxyterminal propeptide of type I procollagen
PINP	aminoterminal propeptide of type 1 procollagen
Pyr	pyridinoline
QALY	quality-adjusted life-year
QCT	quantitative computed tomography
QL	quality of life
RCT	randomized controlled trial
RIA	radioimmuno assay
RM	repeated measures
RR	risk ratio
SD	standard deviation
SGH	salivary gland hypofunction
SHBG	sex hormone-binding globulin
TGF-β	transforming growth factor beta
TMJ	temporo-mandibular joint
TNFα	tumor necrosis factor alpha
TRACP5b	tartrate-resistant acid phosphatase 5b-isoform
TSH	thyroid stimulating hormone
VTE	venous thromboembolism
WEST	Women´s Estrogen for Stroke Trial
WHI	Women´s Health Initiative Study

Abstract

Osteoporosis is characterized by reduced skeletal mass and deterioration of the microachitecture of the skeleton. Consequently, skeletal fragility and risk of fracture increase (Anonymous 1993). Thirty percent of 65–70-year-old women have osteoporosis, and after the age of 80 its prevalence increases up to 70 %. Postmenopausal women with osteoporosis seem to be at an increased risk of cardiovascular events. Furthermore, the deterioration of oral health, as shown by attachment loss of teeth, is proportional to the severity of osteoporosis. Osteoporosis can be treated with many different forms of medication, but data on the efficacy and safety of estrogen treatment comes mainly from early postmenopausal women.

We randomized 90 elderly osteoporotic women between 65 and 80 years of age to receive hormone therapy (HT) (a continuous combination of 2 mg oral estradiol plus 1 mg norethisterone acetate) or 10 mg of alendronate daily or their combination for two years and compared the treatments with regard to their effects on bone mineral density (BMD) and turnover, and two surrogate markers of the risk of cardiovascular diseases (CVDs), C-reactive protein (CRP) and E-selectin, as well as oral health. The effect of HT on health-related quality of life (HRQoL) was studied in a population-based cohort of 1663 postmenopausal women, mean age 68 years, out of which 585 women were estrogen users and 1078 were non-users.

BMD was measured by dual-energy X-ray absorptiometry (DXA) at 0, 12 and 24 months. Urinary N-telopeptide (NTX) of type I collagen, a marker of bone resorption, and serum aminoterminal propeptide of human type I procollagen (PINP), a marker of bone formation, were assayed every six months of treatment. Two surrogate markers of CVD risk, serum CRP and E-selectin, were assayed at 0, 6, and 12 months. Dental, periodontal and intra- and extraoral status, saliva analyses, panoramic tomography of the jaws, buffering capacity, oral yeasts and gingival crevicular fluid (GCF) matrix metalloproteinase (MMP)-8 levels were studied to evaluate oral health status and for mouth symptoms a structured questionnaire was used. The HRQOL was measured by means of a generic, comprehensive, 15-dimensional, standardized, self-administered questionnaire.

Lumbar spine BMD increased similarly in all treatment groups, ranging from 6.8 % to 8.4 % at 12 months and from 9.1 % to 11.2 % at 24 months. Only HT increased femoral neck BMD significantly at both 12 (4.9 %) and 24 months (5.8 %). At the latter time point the HT group differed significantly from the other groups

(+3.3 % for the alendronate group at 12 months; + 2.7 % for the combination group at 24 months). HT reduced bone marker levels of NTX by 60.2-62.7 %, this being somewhat less than that with alendronate alone (72.4-76.1 %, $p=0.047$) or the combination (78.1-80.4 %, $p<0.0001$-0.0069), and the reductions in PINP levels were 53.6-59.8%, 73.0-75.0% ($p<0.001$) and 67.0-71.5% ($p<0.0001$), respectively.

Oral HT increased serum CRP levels by 76.5% at 6 months ($p<0.001$) and by 47.1% at 12 months (NS). The simultaneous rises in serum sex hormone-binding globulin (SHBG) concentrations suggested the changes to be a sign of non-specific stimulation of hepatic protein synthesis by HT. Oral HT decreased serum E-selectin levels by 24.3 % ($p<0.001$) at 6 months and 30.0 % ($p<0.001$) at 12 months. Alendronate did not have any effect and did not block the effect of HT on these surrogate markers of CVD risk.

Alendronate caused a decrease in the resting salivary flow rate (19 %, $p< 0.05$) and tended to increase GCF MMP-8 levels. Otherwise, the treatments did not have any effect on the parameters of oral health.

HT significantly improved the HRQoL of elderly postmenopausal women as regards the dimensions of usual activities, vitality and sexual activity, but the overall improvement in HRQoL was neither statistically significant nor clinically important.

In conclusion, in elderly postmenopausal women HT is effective in the treatment of osteoporosis, but it does not improve the overall quality of life or oral health. HT has divergent effects on markers of the risk of cardiovascular diseases. Given all the potential risks of CVD, thromboembolic events and cancer associated with HT, bisphosphonates might be the first option when starting the treatment of postmenopausal osteoporosis in old age.

Introduction

Osteoporosis is characterized by reduced skeletal mass and deterioration of the microachitecture of the skeleton. Consequently, skeletal fragility and risk of fracture increase (Anonymous 1993).

Osteoporosis may lead to especially wrist, vertebrae and hip fractures. After menopause estrogen deficiency results in increased bone resorption and a reduction in bone mass. Thirty percent of 65–70-year-old women have osteoporosis, and after the age of 80 its prevalence increases up to 70 % (Melton 1995), with 62 % of osteoporotic women having at least one osteoporotic fracture. In elderly women, 90 % of hip fractures are attributable to osteoporosis. Osteoporosis is an underdiagnosed and also an untreated disease, which through fractures increases morbidity, mortality and loss of independence.

According to an American cost-benefit analysis, examination and treatment of osteoporosis are most profitable when directed to 65–70-year-old people (Black 1995). Osteoporosis can be treated with many different medications, for example bisphosphonates or estrogens, but these treatments have not been compared previously in elderly women.

Recent observations suggest that postmenopausal women with osteoporosis are at an increased risk of CVD that is proportional to the severity of osteoporosis at the time of diagnosis. Treatment of postmenopausal osteoporosis should therefore include consideration of measures to prevent cardiovascular outcomes (Tanko *et al* 2005). Consequently, studies in which different treatments of osteoporosis (e.g. HT and bisphosphonates) are compared regarding their effects on surrogate markers of CVD, are well based.

Postmenopausal women with osteoporosis are at an increased risk of attachment loss of teeth, which risk may be attenuated by the use of estrogen replacement therapy (ERT) (Grodstein *et al* 1998, Payne *et al* 1999, Ronderos *et al* 2000). HT has been reported to ameliorate dry mouth feelings (Laine and Leimola-Virtanen 1996, Leimola-Virtanen *et al* 1997, Friedlander 2002, Eliasson *et al* 2003) and increase the salivary flow rate (Laine and Leimola-Virtanen 1996). In an osteoporosis study, combination treatment with HT, alendronate and calcium significantly increased the salivary flow rate (Yalcin *et al* 2005).

The latest evidence from randomized trials has shown several health risks related to HT use, with breast cancer and thromboembolic events being the most important ones (Hlatky *et al* 2002, Rossouw *et al* 2002). Postmenopausal women, making a decision on whether or not to use HT, may however consider well-being to

be a more important factor than the reported health risks. In recent trials, the impact of HT on quality of life has remained unclear. In the heart and estrogen/progestin replacement study (HERS) HT had only a limited effect on HRQoL in elderly women without vasomotor symptoms (Hays *et al* 2003).

Women with severe osteoporosis respond to antiresorptive bisphosphonate therapy by increasing BMD. Consequently, the incidence of fractures is decreased by 40-50%. This decreases pain and results in better mobilisation, social well-being and quality of life (Devogelaer 1998, O'Connell 1999, Epstein 2000).

The purpose of this study was to compare two treatments of osteoporosis with different mechanisms of action, i.e. HT and bisphosphonate, alendronate, in elderly women, separately and in combination, with respect to their effects on bone, oral health, and surrogate markers of cardiovascular diseases, and to examine the effect of HT on HRQoL of elderly women.

Review of the literature

1. Osteoporosis

1.1. Definition of osteoporosis

Bone remodeling constitutes the resorption of old bone and its replacement with new bone. A chronic imbalance in the bone remodeling process, with resorption exceeding formation, results in osteoporosis, which is characterized by reduced skeletal mass and deterioration of the microachitecture of the skeleton. Consequently, skeletal fragility and risk of fracture increase (Anonymous 1993). Osteoporosis is defined by WHO criteria as bone mineral density (BMD) 2.5 SD or more below the mean of a reference population of young premenopausal women. Osteoporosis is currently defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture (NHI Consensus Development Panel on Osteoporosis 2001). Bone strength primarily reflects the integration of bone quality and bone density. Bone quality refers to architecture, turnover, damage accumulation and mineralization.

Bone turnover is maintained by two groups of cells. Activated osteoclasts wander to a resorption site and dig pits in mineralized matrix by secreting enzymes and hydrochloride acid. In the consequent synthesis phase osteoblasts refill the pits with new bone, which mineralizes, and a new osteon is completed. Osteoclastic activity is regulated by the action of sex steroids, especially estrogens, and local factors. Coordination with osteoblasts is normally maintained such that there is no net change in bone mass during early adult life (Bell 2001).

1.2. Epidemiology of osteoporosis and fractures

In the menopause, estrogen deficiency results in increased bone resorption and a reduction in bone mass. Thirty percent of 65–70-year-old women have osteoporosis, and after the age of 80 its prevalence increases up to 70 % (Melton 1995), with 62 % of osteoporotic women having at least one fracture. Vertebral fractures are the most common osteoporotic fracture, with one in every three women experiencing it in their lifetime (Cummings and Nevitt 1989), but 50 % of them occur without symptoms. The most serious fracture is hip fracture, which carries a high risk of mortality, with 10-20 % of women dying earlier than expected for age within the first year after the fracture (Cummings and Melton 2002). The lifetime risk of hip fracture at 50 years of age is 17.5% for women, and 6 % for men (Lips 1997). The incidence of wrist fracture increases up to the age of 60-65 years and it plateaus thereafter. Most hip fractures occur after the age of 70 years, when age-associated fragility, falls, and bone

loss are the most important risk factors in the development of such fractures (Hui *et al* 1988). Because the world´s population is ageing, the frequency of hip fractures is increasing by 1-3 % per year in the most areas of the world (Cummings and Melton 2002).

Age is an independent risk factor for fractures. A reduction of 1 SD in BMD (Fig.1), and ten years' ageing, each double the risk of fracture independently of one another.

In Finland there are estimated to be 400 000 patients with osteoporosis, and every year there are 40 000 new fractures, 7000 of them being hip fractures (Alhava 2004).

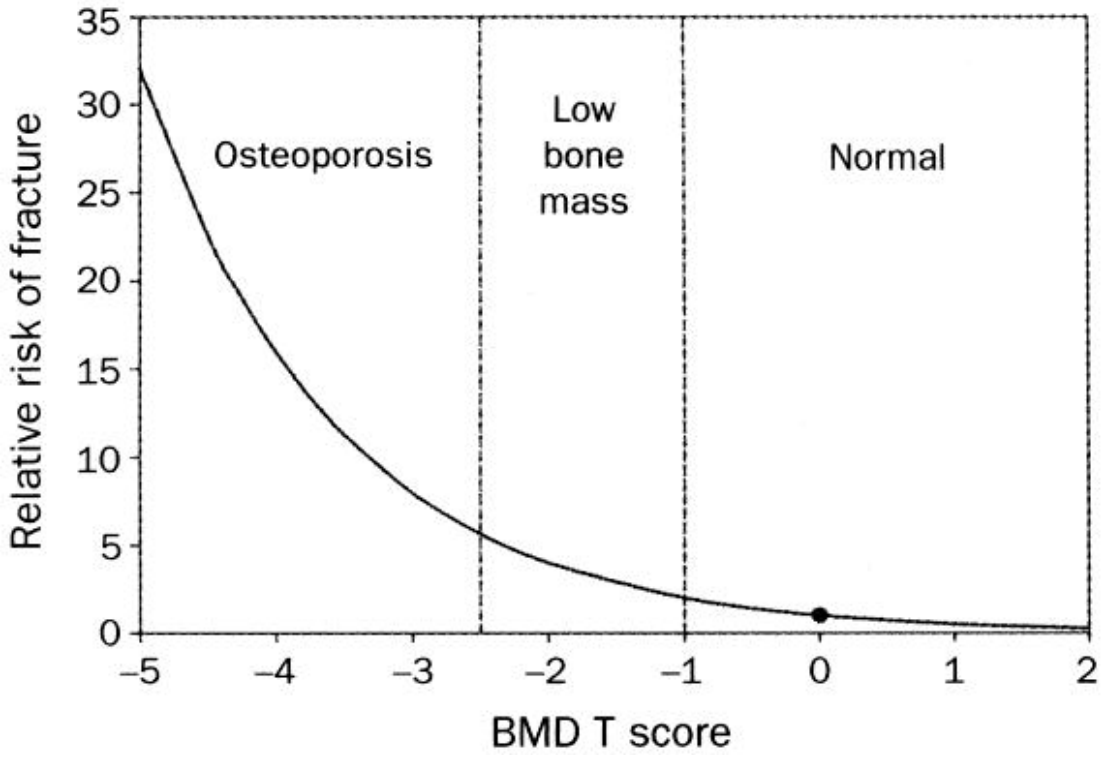


FIGURE 1. Relationship between bone mineral density (BMD) and fracture risk based on a doubling of risk with each SD decrease in BMD (most epidemiological studies have reported relative risks between 1.5 and 2.5 per SD of BMD). A relative risk of 2.0 indicates the risk is twice as high compared with the reference value. T scores between -1.0 and -2.5 indicate osteopenia, and values lower than -2.5 indicate osteoporosis.

1.3. Assessment of bone

1.3.1. Bone mineral density

Dual-energy X-ray absorptiometry (DXA) and quantitative computed tomography (QCT) are the best biophysical ways to assess bone in clinical practice. DXA allows a greater variety of measurement sites. For repeated measurements, DXA provides good precision (error 1-2%) at a low radiation burden (<1 μSv), stable calibration and a

short scanning time (2-5min). Repeated measurements permit estimation of the rate of bone loss in untreated patients and give useful information about treatment effect. The metabolically more active trabecular bone in the lumbar spine changes faster after menopause and during treatments of osteoporosis than the cortical bone in the hip. Thus, for repeated measurements the lumbar spine appears to be the most sensitive skeletal site, but it is more sensitive than the hip to artefacts (Wahner 1989). In clinical practice DXA is the most commonly used technique for measurement of areal bone density (g/cm^2). Despite its many advantages, there are some problems with DXA. Besides the vertebral body the measurement also captures the arches and spinous processes. Aortic calcification, radio-opaque contrast media, metallic objects, distribution of fat tissue, previous vertebral fractures, scoliosis, osteomalacia or osteoarthrosis may affect the results at the lumbar spine (Kröger and Reeve 1998).

QCT is the only alternative to measure true volumetric bone density (g/cm^3), and trabecular and cortical bone areas can be measured separately. Availability, expenses and higher radiation dose (50 μSv or more) are the limits of this technique in clinical practice (Wahner 1989).

1.3.2. Bone turnover markers

There are several biochemical markers that can be used to quantify bone metabolism. These assays measure in serum or in urine enzymes or matrix proteins synthesized or degraded by bone cells. They can be divided into those measuring bone formation and those quantifying bone resorption.

1.3.2.1. Markers of bone formation

Alkaline phosphatase

The common form of alkaline phosphatase (ALP) is a cell-membrane-associated enzyme expressed by the liver, bone, kidneys and placenta. In adults the liver and bone are the major sources of ALP. In bone, ALP is derived from osteoblasts and their precursors, and it has a role in bone mineralization. ALP may increase the local concentration of inorganic phosphate, destroy local inhibitors of crystal growth, transport phosphate and act as a calcium-binding protein. To be used as a marker of bone formation, the bone-specific isoform (bone ALP) has to be distinguished from the liver-specific-isoform (*Behr and Barnert* 1986).

Osteocalcin

Human osteocalcin (OC) is a 49-residue polypeptide of γ-carboxyglutamic acid (Gla), which is synthesized by mature osteoblasts during the mineralization phase of bone remodeling in a vitamin K-dependent carboxylation process. Vitamin D modulates the OC-gene (Stein *et al* 1996). OC is also found in other calcified tissues such as dentin

and calcified cartilage. In the bone it forms about one per cent of the organic matrix. OC is released into the circulation during the bone formation, and it is filtered in the kidneys. However, as OC is incorporated into bone extracellular matrix, some of the circulating OC might originate from degrading bone. Thus circulating OC may reflect bone turnover rather than bone formation alone (Riggs *et al* 1986).

Type I collagen propeptides

Type I collagen is synthesized by osteoblasts from type I procollagen precursor proteins. These precursors have large extension domains at both ends. While type I collagen is being synthesized, the type I aminoterminal and carboxyterminal propeptides, PINP and PICP respectively, are enzymatically removed and released into the circulation (Calvo *et al* 1996). As bone is the major organ synthesizing type I collagen, PINP and PICP reflect bone formation (Delmas 1992, Ebeling *et al* 1992). PINP and PICP are degraded in the liver and elevated serum levels have been measured in patients with chronic liver disease (Guanabens *et al* 1994). PINP and PICP can be measured by immunoassays (Melkko *et al* 1990, Melkko *et al* 1996).

1.3.2.2. Markers of bone resorption

Bone resorption markers reflect collagen degradation and proteolysis of collagen by osteoclasts.

Pyridinoline cross-links

Collagen molecules are held together by hydrogen bonds and pyridinium cross-links. When collagen degrades, these cross-links (pyridinoline [Pyr] and deoxypyridinoline [Dpy]) are released into the circulation, and then excreted into urine. Because bone is the major reservoir of type I collagen, and it turns over faster than most major connective tissues, pyridinolines in adult urine are mostly derived from bone and thus reflect bone resorption (Eyre 1997).

Tartrate-resistant acid phosphatase

Tartrate-resistant acid phosphatase is an enzyme, the 5b-isoform (TRACP5b) of which is expressed in high amounts in osteoclasts. During bone degradation TRACP5b is released into the circulation. It has been shown to be present in elevated concentrations in patients with metabolic bone diseases. It seems to be a relatively sensitive and specific marker of bone resorption. It reflects both cathepsin-K- and MMP-mediated bone degradation. During bisphosphonate therapy serum TRACP5b concentrations decrease (Halleen *et al* 2001).

Cross-linked telopeptides of type I collagen

When type I collagen is degraded, it is split into several fragments. Cathepsin-K and MMPs take part in this process. Two fragments have been characterized in the carboxyterminal end of type I collagen. The first is a cross-linked carboxyterminal

telopeptide of type I collagen (ICTP) and the second a C-terminal cross-linked telopeptide of type I collagen (CTX). ICTP is a larger molecule than CTX (Garnero *et al 2003*). There is another type I collagen telopeptide (NTX) in the aminoterminal end. It is thought that cathepsin-K releases mostly CTX and NTX, and MMPs ICTP (Garnero and Delmas 1998, Garnero *et al* 2003). CTX and NTX can be measured in urine or serum by immunoassays (Risteli *et al* 1993). These markers have been shown to be present in elevated concentrations in patients with metabolic and metastatic bone diseases (Tähtelä and Tholix 1996, Fohr *et al* 2003, Garnero *et al* 2003). During antiresorptive treatment, serum and urinary concentrations of CTX and NTX decrease (Christgau *et al* 2000).

Changes in bone turnover can be measured by means of the above mentioned biochemical markers within a few months of beginning treatment.

2. Consequences of menopause

2.1. Climacteric symptoms

Menopause results in a decrease in serum estradiol concentrations and cessation of natural menstruation. It occurs on average at the age of 51 years (range 45-55 years) in Western countries (Oldenhave *et al* 1993). Estrogen deficiency due to ovarian failure causes climacteric symptoms, such as hot flushes, night sweats, irritability, depression, headache, palpitations, sleeping disorders and vaginal dryness (Stearns *et al* 2002). At least 75 % of all women have these symptoms at menopause, usually for two years, and 25-50% of symptomatic women have them for more than 5 years (Oldenhave and Netelenbos 1994); 20 % of women have them for 15 years (Stearns *et al* 2002). Among elderly women in the HERS trial (mean age 67 years) 15.7% still had hot flushes. Despite of the high prevalence of the symptoms, their pathophysiology remains mainly unknown. A decline in estrogen concentrations might lead to alterations in brain neurotransmitters and to instability in the hypothalamic thermoregulatory set - point (Stearns *et al* 2002). The symptoms are more common in smokers and in underweight and sedentary women (Stearns *et al* 2002).

Urogenital symptoms caused by estrogen deficiency and atrophic urogenital epithelia are complained of by 10-40 % of postmenopausal women (Cardozo *et al* 1998). The most common complaints are vaginal discharge, itching, burning, dyspareunia, and recurrent urinary and vaginal infections (Raz and Stamm 1993, Cardozo *et al* 1998).

2.2. Effects on bone

The velocity of bone loss increases during ageing. BMD begins to decrease in both sexes before 30-35 years of age, but before menopause the net loss is slow as a result of slow remodeling in estrogen-sufficient women. In bone, estrogens inhibit bone resorption, to a great extent through receptors in osteoblasts, by down-regulating the expression of bone- resorbing cytokines, such as interleukins (IL)-1α, IL-1β, IL-6 and tumor necrosis factor-α (TNF-α). Lack of estrogens leads to increased activity of these cytokines. In menopause, circulating estrogen concentrations fall rapidly and osteoclastic activity accelerates, outstripping the attempts of osteoblasts to keep pace. Especially in trabecular bone the interval between remodeling cycles shortens and at the osteon level both bone resorption and formation are accelerated, resorption more than formation (Riggs *et al* 2002). In late postmenopausal women (age more than 60 years), low E2 levels are associated with low trabecular and cortical BMD (Khosla *et al* 2005). Similar to findings in men, the threshold for estrogen deficiency in cortical bone in women appears to be lower than that in trabecular bone (Khosla *et al* 2005). BMD decreases by approximately 1.5 % each year during the first 3 years after menopause, followed by an annual loss of 0.3-0.4 % after that (Komulainen *et al* 1999). The net result is bone loss of up to 50 % in trabecular bone and 30 % in cortical bone by age 80 in women (Seeman 2004). Bone resorption markers increase in menopause, and increased levels seem to be associated with an increased fracture risk in elderly women (Looker *et al* 2000).

2.3. Effects on risks of cardiovascular diseases and their surrogate markers

CVDs are rare in premenopausal women compared with age-matched men (Barrett-Connor and Bush 1991, Isles *et al* 1992), but after onset of menopause the occurrence of CVD events rises and by the age of 70 years it is equal in women and men (Carr 2003).

The concentrations of total cholesterol, LDL, and VLDL cholesterol as well as triglycerides seem to increase with age in both sexes. In longitudinal studies atherogenic changes in lipid and lipoprotein profiles have been observed within two years preceding the menopause (Jensen *et al* 1990, Do *et al* 2000). Increases of both total and LDL cholesterol concentrations have been observed during menopause, and a shift to smaller and denser and potentially more atherogenic LDL particles has been related to menopause (Campos *et al* 1988), but data on HDL cholesterol have been inconsistent. A decline in HDL2 and a rise in HDL3 may result in an unfavorable net effect (Matthews *et al* 1994).

The risk of impaired glucose metabolism, and abdominal obesity, increase after menopause. They have been shown to be independent risk factors for coronary heart diseases (CHD), cardiovascular mortality and future diabetes (Carey *et al* 1997,

Wajchenberg 2000, Hu *et al* 2004). Hypertension and endothelial dysfunction are also likely to develop after menopause, increasing the risk of CVDs (Gambacciani *et al* 2002).

Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis and they mediate atheroma development from initial leukocyte recruitment to the eventual rupture of an unstable atherosclerotic plaque (Blake and Ridker 2001, Ambrose and Martinez 2002). Elevated plasma levels of markers of the inflammatory cascade (P-selectin, interleukins (IL), tumor necrosis factor alpha [TNFα], soluble intercellular adhesion molecule-1, and CRP) seem to predict plaque rupture. Synthesis of CRP in the liver is induced by IL-1 and –6, and minimal changes can be measured immunochemically with highly sensitive assay using monoclonal antibodies. It seems to be the most powerful inflammatory marker of future cardiovascular risk, and to have a direct proinflammatory effect (Ridker *et al* 1998, Lagrand *et al* 1999, Ridker *et al* 2000, Blake and Ridker 2001, Danesh *et al* 2004). At menopause the plasma levels of E-selectin increase (Kennedy *et al* 1999), but ageing in itself does not have any effect on CRP levels in postmenopausal women (Sites *et al* 2002). Final occlusion of an artery results when circulating macrophages and lymphocytes are trapped from on the vascular wall by adhesion molecules, e.g. E-selectin (Gearing and Newman 1993). Thus, E-selectin may play a role in the development of atherosclerosis (Farzati *et al* 2002), and high plasma levels may be associated with an increased risk of CVD.

Plasma SHBG is sythetized in the liver and it regulates the bioavailable fraction of steroids and also their access to target cells (Kahn *et al* 2002). Estrogen and thyroid hormones increase, while androgens decrease the synthesis of SHBG (Nachtigall *et al* 2000). Hyperinsulinemia, insulin like growth factor-1 and hyperandrogenism are associated with low SHBG levels (Hogoveen *et al* 2002, Kalme *et al* 2003). Decreased levels of SHBG are explained by estrogen deficiency after menopause (Sarrel 2002).

Calcification is an active process in CVDs, especially in coronary arteries. Epidemiological evidence has shown the coexistence of vascular calcification with both atherosclerosis and osteoporosis, and hyperlipidemia and atherogenic phospholipids in vascular calcification. Many bone regulatory factors have been shown to be present in calcified atherosclerotic lesions, and regulate mineralization in both bone and vasculature and may account for the co-existence of osteoporosis and atherosclerotic calcification (Tintut and Demer 2001). In the placebo group in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial an inverse association between BMD and the severity of atherosclerosis was shown in postmenopausal women (Tanko *et al* 2005). Osteoporotic women were at an increased risk of cardiovascular events, both coronary events and stroke. The association was not

explained by the usual surrogate markers of CVD, e.g. CRP or lipids, but as linking factors the authors suggested the regulators of bone turnover, e.g. matrix GLA protein, osteoprotegerin, and osteocalcin, proinflammatory cytokines such as IL-6 and TNF-α, oxidized lipids or NO synthase (Tanko *et al* 2005).

2.4. Effects on oral health

The oral status of premenopausal women is better than that of menopausal women (Yalcin *et al* 2005). Estrogen deficiency after menopause may cause oral health problems, e.g. tooth loss (Grodstein *et al* 1998). Xerostomia and salivary gland hypofunction (SGH) increase in the menopausal period and are prevalent in elderly populations, more in women than in men, causing much discomfort and even difficulties in eating (Närhi *et al* 1999). Buccal mucosa and salivary gland tissue contain estrogen receptors and are thus estrogen-responsive tissues (Leimola-Virtanen *et al* 2000). Dry mouth symptoms caused by insufficient salivary secretion due to estrogen deficiency, concomitant diseases and medication are an increasing oral health problem with advanced age. The salivary flow rate has been shown to decrease in the menopausal period (osteoporotic women aged 50.7 years compared with non-menopausal control women aged 42.4 years) but to increase significantly ($p=0.03$) after osteoporosis combination treatment of HT (conjugated estrogen (CEE) 0.0625mg plus MPA 5mg) with alendronate and calcium supplementation in early postmenopausal women (Yalcin *et al* 2005). Burning or painful mouth is a condition that elicits a burning sensation in the oral cavity. When no obvious somatic pathology can be found, the condition is called burning mouth syndrome (BMS). Among 50- to 58-year-old women, 8.2 % suffer from BMS and 19.9 % from dry mouth symptoms. Despite of HT, climacteric symptoms, smoking and antidepressants are risk factors for BMSs, which are a common complaint in elderly women (Tarkkila *et al* 2001). Osteoporosis correlates with the loss of alveolar bone and increases the risk of attachment loss of teeth (Wactawski-Wende *et al* 1996, Payne *et al* 1999, Reinhardt *et al* 1999, Wactawski-Wende *et al* 2005).

Oral health status can be evaluated by dental, periodontal and intra- and extra-oral status, saliva and gingival crevicular fluid tests, panoramic tomography of the jaws (OPTG), by measuring the buffering capacity of the saliva and by looking for oral yeasts.

2.5. Effects on health-related quality of life

Estrogen deficiency at menopause causes multiple physiologic changes, which affect quality of life (QoL) with the interplay of different biological, cultural, social and psychological factors. Standardized questionnaires are used to study health- related

quality of life (HRQoL). Generic HRQoL tests cover all aspects of health, whereas disease-specific tests concentrate on the complaints typical of some particular disease.

Menopausal women have been found to have worse QoL scores than younger women, with a 3.5-fold risk of psychosocial impairment, a 5.7-fold risk of physical disorders, a 3.2-fold risk of sexual disorders and a 10.6-fold risk of vasomotor disorders (Blumel *et al* 2000). Menopause is associated with loss of libido, dyspareunia, and anorgasmia (Bachmann 1995). Forty-six percent of women with hot flushes report a reduced libido (Chiechi *et al* 1997). In other studies the effects of menopause on QoL have been of minor importance (Avis and McKinlay 1991, Ekström and Hovelius 2000).

In elderly women, quality of life scores have been reported to decline significantly as regards physical function, mental health, and energy/fatigue over 3 years (HERS) (Hlatky *et al* 2002).

At least 75 % of all women have hot flushes at menopause, reducing the QoL, and 25-50 % have them for more than 5 years (Oldenhave and Netelenbos 1994). In particular, underweight and sedentary women with little or no exercise, and smokers, are at an increased risk of hot flushes (Stearns *et al* 2002). Symptoms and complaints differ between populations and cultures, and a low educational level and low socioeconomic status are risk factors for hot flushes (North American Menopause Society 2004).

Questionnaires

The most frequently used generic measure in HT studies has been the Nottingham Health Profile (NHP). Other options have been 15D, RAND-36, Short Form Survey (SF)-36, Sickness Impact Profile (SIP), Medical Outcomes Study (MOS), Health Utility Index (HUI) and EuroQoL (EQ-5D) (Hunt *et al* 1981, MacKeigan and Pathak 1992). The Women´s Health Questionnaire and Menopause Rating Scale are disease-specific measures, designed for climacteric symptoms. The generic HRQoL measures are better established than the disease-specific measures, but the disadvantage of the generic tests is their lack of sensitivity to health changes in specific conditions. They may detect benefits and adverse effects of treatments that are not anticipated. The disease-specific measures are considered more acceptable, but their validity is not as well established (Sintonen *et al* 2003).

The 15D questionnaire is a generic self-administered and standardized HRQoL measure, which is well documented in terms of reliability, validity, discriminatory power and responsiveness to health changes (Kauppinen *et al* 1998, Sintonen 2001). It contains 15 multiple choice questions. Each question represents one health-related dimension, concerning mobility, vision, hearing, breathing, sleeping, eating, speech, elimination, usual activities, mental function, discomfort and symptoms, depression, distress, vitality and sexual activity. NHP is a generic self-administered measure with

established reliability. It consists of statements on how a subject feels or functions. The 38 statements are divided into six dimensions covering energy, sleep, pain, emotional reactions, social isolation and physical mobility (Hunt *et al* 1981). The NHP and 15D questionnaires gave similar results in assessment of HRQoL of women on HT (Sintonen *et al* 2003).

Based on the structure of the questionnaire, HRQoL measures can be categorized into profiles and single index measures. The profiles comprise multiple dimensions representing different aspects of health. Every dimension is scored separately (MacKeigan and Pathak 1992). The scores from all the dimensions can be summed up to obtain a single index number varying from 0 (death) to 1 (full health) (Brazier *et al* 1998).

3. Hormone therapy

3.1. General principles

Menopausal complaints have been treated with estrogens, alone or in combination with progestogen, for more than 50 years (Barrett-Connor 2003). Only estrogen is needed to alleviate climacteric symptoms, but with an intact uterus progestin is needed because of the increased risk of hyperplasia and endometrial cancer (Manson and Martin 2001). Estrogen regimens can be oral, transdermal, intranasal or subcutaneous. Progestogen is given sequentially or continuously either orally, transdermally or via the intrauterine route (North American Menopause 2004). In Finland 22 % of all postmenopausal women used HT in 2002. Most commonly, use was at 55-56 years of age (Salmi *et al* 2004). Between Finnish HT users and non-users there are no socioeconomic differences under 55 years of age, but older educated women use HT more frequently than less educated women (Topo *et al* 1999).

In the United States 35-40 % of postmenopausal women of 50-74 years of age use HT, but 50-60 % use it for less than one year (Keating *et al* 1999, Ettinger. 2003). Only 20 % of women use HT for 5 years or more in the USA (Brett and Madans 1997). HT use is common among well educated postmenopausal women. Cultural and socio-demographic factors, such as region and education, may be more strongly associated with use of HT than clinical factors (Keating *et al* 1999, Genazzani *et al* 2002). In Finland, HT use has decreased by 12 % for estradiol-only preparations, by 21 % for cyclic HT and by 8 % for continuous combined HT during the past few years (Erkkola 2004).

3.2. Effects on bone

Estrogen plays pivotal roles in the function and maintenance of the skeleton, including the bone-forming osteoblasts. The functions of E2 are largely mediated through two distinct estrogen receptor (ER) isoforms, ERalpha and ERbeta, both of which are expressed in osteoblasts (Monroe *et al* 2003). Estrogen reduces bone resorption by decreasing serum levels of osteoclast-stimulating cytokines, interleukins and tumor necrosis factor-α, and by up-regulating transforming growth factor (TGF)-ß, which inhibits bone resorption by decreasing the activity of osteoclasts and by increasing their apoptosis (Manolagas 2000). Estrogens prevent postmenopausal bone loss, improve bone density by 5-10 % over 1-3 years (Genant *et al* 1989, Lindsay and Tohme 1990, Schneider *et al* 1997, Recker *et al* 1999, Wells *et al* 2002), and decrease the risk of vertebral and non-vertebral fractures, including hip fractures, in populations of postmenopausal women not selected on the basis of osteoporosis (Rossouw *et al* 2002, Cauley *et al* 2003). They also decrease the risk of vertebral fractures in established osteoporosis (Lufkin *et al* 1992). In a meta-analysis of randomized controlled trials of HT a statistically significant reduction in non-vertebral fractures was noted (Torgerson and Bell-Syer 2001). In a cohort study, hormone therapy effectively prevented hip fractures among women older than 75 years (Cauley *et al* 1995). In the WHI population, not selected as regards BMD or fracture history, HT reduced the risk of vertebral, hip and non-vertebral fractures in women with a mean age 63 years, the absolute risk reductions per 10 000 person-years being 5 fewer hip, 18 fewer wrist/lower arm and 47 fewer total fractures (Cauley *et al* 2003). Estrogens combined with progestins may increase vertebral BMD more than estrogens alone, with the effect being most pronounced during the first two years of HT (Christiansen and Riis 1990, Anonymous 1996, Speroff *et al* 1996). Some progestins have a more powerful effect on bone than others (Hosking *et al* 1998).

3.3. Cardiovascular benefits and risks

Estrogen has several favorable effects, that may be protective against CVD, serum lipids and lipoproteins having been most widely studied (The Writing Group for the PEPI trial 1995, Godsland 2001, Davison and Davis 2003). Epidemiological data have shown a close association between serum lipoproteins and CVD risk (Rich-Edwards *et al* 1995). High levels of LDL and low levels of high density lipoproteins (HDL) are regarded as predictors of such risks (Margolis 1990). Estrogens decrease serum levels of total and LDL cholesterol, lipoprotein (Lp(a)), and increase that of HDL cholesterol (Lobo 1991, Barrett-Connor and Miller 1993). The lipid and lipoprotein responses to HT depend on the type and dose of estrogen and the route of administration (Godsland 2001).

Estrogens have a favorable effect on the vascular wall, by increasing the production of vasodilatory prostacyclin (Ylikorkala *et al* 1986, Vane *et al* 1990) and nitric oxide (NO) (Cicinelli *et al* 1997), which also inhibits platelet aggregation (Moncada *et al* 1991). Endothelin-1 (ET-1) is a vasoconstrictive peptide produced by endothelial and smooth muscle cells (Masaki 1993). Estrogens decrease the serum levels of ET-1 in postmenopausal women (Ylikorkala *et al* 1995, Wilcox *et al* 1997). Long-term HT has shown a protective effect on age-related thickening of the intima-media of carotid artery after the menopause (Tremollieres *et al* 2000).

The effects of estrogens on glucose metabolism and insulin sensitivity are conflicting (Barrett-Connor and Laakso 1990, Espeland *et al* 1998, Raudaskoski *et al* 1999, Karjalainen *et al* 2001). In the recent clinical trial CEE tended to have a protective effect on the incidence of diabetes (HR 0.88, 95 % CI 0.77-1.01) (*Bonds et al* 2006).

Oral estrogen plus progestogen treatment has been associated with increased levels of CRP (Cushman *et al* 1999, Ridker *et al* 1999, van Baal *et al* 1999, Luyer *et al* 2001, Prelevic *et al* 2002), although not in all studies (Zanger *et al* 2000, Oger *et al* 2001). In contrast, transdermal HT reduced CRP levels (Vehkavaara *et al* 2001). The type and route of the estrogen component may be most significant as regards the elevations in CRP concentrations, but progestin may also have an influence (Sattar *et al* 1999, Zanger *et al* 2000). Women using HT orally (Cushman *et al* 1999, Zanger *et al* 2000, Luyer *et al* 2001) or transdermally (Oger *et al* 2001, Vehkavaara *et al* 2001, Farzati *et al* 2002) are characterized by having 18-35 % lower serum E-selectin levels than non-users. Transdermal HT has been reported to have no effect on E-selectin levels (Oger *et al* 2001).

Oral ERT elevates the concetrations of SHBG (Samsioe 2002), but transdermal ET has no effect (Samsioe 2002). The effect of the progestogen on SHBG depends on the androgenicity of the treatment. Androgenic progestogens, e.g. NETA, decrease the synthesis of SHBG, whereas cyproterone acetate and dydrogesterone have no effect on the concentrations (Nugent *et al* 2003).

Table 1. The effects of estrogens on CVD risk factors

(↑increase, ↓ decrease, - no change).

Risk factor	Oral	Transdermal
C-reactive protein	↑	↓, -
E-selectin	↓	↓, -
Lipids and lipoproteins		
Total cholesterol	↓↓	↓
LDL cholesterol	↓↓	↓
HDL cholesterol	↑↑	↑, -
Triglycerides	↑	↓, -
Lp(a)	↓	-
Endothelial cell function		
Prostacyclin	↑	-
Nitric oxide	↑	↑
Endothelin-1	↓	↓
Endothelium vascular resistance	↓	↓
Homocysteine	↓ , -,↑	↓, -
Insulin sensitivity	↑ , -	↑, -

3.3.1. Coronary heart disease

In recent studies no cardiovascular benefits, or even adverse effects, have been reported in connection with HT (Grady *et al* 2002, Rossouw *et al* 2002). In the WHI study the risk of CHD in the estrogen group remained unchanged (hazard ratio [HR] 0.91, 95 % CI 0.75-1.12), but it was increased (HR 1,29, 95 % CI 1.02-1.63) in the estrogen-progestin group. On the other hand, the total CVD event rate, including stroke, was increased in the estrogen group (HR 1.12, 95 % CI 1.01-1.24) in comparison with the placebo group (The Women's Health Initiative Steering Committee 2004).

3.3.2. Stroke

In the WHI study the risk of stroke increased in women with a mean age of 63 years in both groups, ERT (HRs 1.39, 95% CI 1.10-1.77) and estrogen-progestin therapy group (HRs 1.41, 95% CI 1.07-1.85) (Rossouw *et al* 2002, The Women's Health Initiative Steering, Committee 2004). Estrogens should not be used for secondary prevention of stroke (Viscoli *et al* 2001), since after an ischemic stroke the risk of recurrence was increased during the first 6 months (relative risk (RR) 2.3, 95 % CI 1.1-5.0), but not significantly over 2.8 years (RR 1.1, 95% CI 0.8-1.4).

3.3.3. Venous thromboembolism

The balance between the coagulation and fibrinolytic systems determines the risk of venous thrombosis. Estrogens have a complex effect on hemostatic factors. They activate fibrinolysis, but still increase the risk of venous thromboembolism (VTE).

The procoagulant changes include increases in factor VII, prothrombin fragments 1 and 2, resistance to activated protein C and decreases in antithrombin III and protein S. On the other hand, the increased D-dimer levels, and reduced levels of fibrinogen and plasminogen activator inhibitor-1 suggest increased fibrinolytic activity (Grodstein *et al* 1996, Conard *et al* 1997, Koh *et al* 1997, Braunstein *et al* 2002).

Current use of HT is associated with an increased risk of venous thrombosis (VTE) (Daly *et al* 1996). In contrast to peroral HT, the effects of transdermal estrogen on hemostatic factors appear to be insignificant with lack of hypercoagulability effect (Koh *et al* 1997, Vehkavaara *et al* 2001). The results of a case control study suggested the odds ratios for VTE in current users of oral and transdermal estrogen replacement therapy (ERT) vs. non-users to be 3.5 (95% CI 1.8-6.8) and 0.9 (0.5-1.6), respectively. The estimated risk for current users of oral vs. transdermal ERT was 4.0 (1.9-8.3) (Scarabin *et al* 2003). Observational studies indicate that postmenopausal use of estrogen increases the risk of deep VTE by a factor of 3.6 (95% CI 1.6-7.8) (Jick *et al* 1996). A meta-analysis of studies of estrogen use and risk of VTE showed a summary relative risk of 2.14 (Nelson *et al* 2002), whereas the data from the 3 randomized controlled trials (RCTs) gave a relative estimate of 3.75 (Humphries and Gill 2003). In the HERS trial the risk of VTE was increased by a factor of 2.7 among elderly women (mean age 67 yr) assigned to receive estrogen-progestin therapy (Manson and Martin 2001). The highest risk is during the first year of use (3.2 additional events per 10 000 women-years) (Nelson *et al* 2002, Humphries and Gill 2003), among women with coagulation abnormalities (Braunstein *et al* 2002), and among women taking high doses of estrogen (Jick *et al* 1996). After the first year the additional events are only 1.2 per 10 000 woman-years (Humphries and Gill 2003).

3.4. Effects on oral health

Buccal mucosa and salivary gland tissue contain estrogen receptors and might thus be estrogen-responsive tissues (Leimola-Virtanen *et al* 2000). HT has been reported to ameliorate dry mouth feelings (Laine and Leimola-Virtanen 1996, Leimola-Virtanen *et al* 1997, Friedlander 2002, Eliasson *et al* 2003).

Nearly 32 % of women in the USA aged 65 to 69 years have no teeth. The risk of tooth loss is 24 % lower in estrogen users than in non-users. Thus, estrogens may reduce tooth loss (Grodstein *et al* 1996).

HT has significantly increased alveolar bone mass compared with placebo, and it tended to improve alveolar crest height (Civitelli *et al* 2002). Severe clinical attachment loss of teeth (11.9 % vs.18.6 %) and alveolar bone loss (20.3 % vs. 34 %) have been reported to be decreased in HT users in comparison to non-users (Grossi 1998).

Osteoclasts resorb bone by secreting acid and proteolytic enzymes into extracellular resorption lacunas. Two major groups of proteinases, MMPs and cysteine proteinases, play the greatest role in degradation of the organic matrix, which is composed mainly of type 1 collagen (Parikka *et al* 2001). Estrogen*s* reduce the depth of resorption pits by decreasing the organic bone matrix degradation activity of mature osteoclasts and by decreasing the activity of cysteine proteinases (Parikka *et al* 2001) and MMPs (Liao and Luo 2001).

3.5. Effects on quality of life

HT has been shown to relieve menopausal symptoms effectively shortly after the start of treatment (McNagny 1999, MacLennan *et al* 2001) and it thereby improves the general well-being of early postmenopausal women (Wiklund *et al* 1992 Daly *et al* 1993, Zethraeus *et al* 1997, Genazzani *et al* 2002, Sintonen *et al* 2003). The impact of HT on generic HRQoL has not been so widely studied, but the results have been promising in early menopause (Wiklund *et al* 1992, Zethraeus *et al* 1997, Genazzani *et al* 2002). At menopause, women (mean age 53 years) on transdermal estrogen combined with norethisterone acetate experienced improvement in all dimensions of the NHP questionnaire, especially as regards sleep and energy dimensions (Wiklund *et al* 1992). In addition, low-dose continuously combined HT has been found to be of value in the enhancement of HRQoL (15D) of relatively young postmenopausal women (mean age 56 years) for whom the relief of menopausal symptoms and control of bleeding are primary objectives of treatment (Ylikangas et al 2005). In more elderly women (mean age 62 years) continuously combined estradiol valerate (E2V) plus MPA has been found to have a positive impact on all dimensions of the 15D and NHP questionnaires, with minimal breakthrough bleeding and limited adverse effects (Sintonen *et al* 2003).

In the WHI study women assigned to use HT (mean age 63 years) experienced fewer sleep disturbances, better physical functioning and less bodily pain than women who were assigned to use placebo (RAND-36). The difference was statistically, but not clinically significant at one year of treatment, but it disappeared with continuation of treatment for an additional two years (Hays *et al* 2003).

The HERS trial showed mixed effects, and women without hot flushes at entry showed declines in physical measures and those with hot flushes showed improvements in emotional measures when assigned to HT (mean age 67 years). The HRQoL, measured by means of several questionnaires (RAND, depressive symptoms on the Burnam screening scale), was impaired in older women and in those with chronic diseases (diabetes, hypertension, chest pain, heart failure). The negative effects on QoL outweighed the positive impact of HT (Hlatky *et al* 2002).

3.6. Malignancies

The Collaborative Group on Hormonal Factors in Breast Cancer has reanalyzed about 90 % of the worldwide epidemiological evidence on the relationship between risk of breast cancer and use of HT. 52 705 women with breast cancer and 108 411 women without breast cancer were analyzed centrally. Thirty-three percent of the women had used HT at some time, and 34% of users had used HT for 5 years or more.. The risk of breast cancer seems to increase with duration of HT. This effect is reduced after cessation of use of HT and disappears in about 5 years (Anonymous 1997). In the WHI study estrogen plus progestin increased the absolute risk of breast cancer by 8 additional cases per 10 000 person-years (HR 1.26, 95 % CI 1.00-1.59) (Rossouw *et al* 2002). Breast cancers tended to be at a more advanced stage during HT vs. placebo at the time of diagnosis (Chlebowski *et al* 2003). In the estrogen-only arm no increased risk of breast cancer was found after seven years' estrogen use vs. placebo (HR 0.80, 95 % CI 0.62-1.04), in exploratory analyses ductal carcinomas were reduced in the CEE group (HR 0.71, 95 % CI 0.52-0.99) (The Women's Health Initiative Steering, Committee 2006).

Endogenous and exogenous estrogen is a risk factor as regards endometrial cancer (Grady *et al* 1995). Estrogen promotes the formation of estrogen receptors and proliferation of the endometrium whereas progesterone down-regulates these receptors and causes secretory changes. This explains why estrogens without progestin increase the risk of hyperplasia and in a minor proportion of patients cause endometrial cancer (Westhoff *et al* 2000, Manson and Martin 2001). A meta-analysis of 29 epidemiological studies showed a 1.4-fold risk of endometrial cancer with less than one year of estrogen use, and a 9.5-fold risk with more than ten years of estrogen use without progestin (Grady *et al* 1995). Combined estrogen and progestin therapy reduces endometrial hyperstimulation and has been found to reduce the rate of atypical endometrial lesions significantly; cyclic progestin therapy seems to be as effective as continuous low-dose progestin (Humphries and Gill 2003).

Recently, estrogen use has been associated with a reduced risk of colon cancer, although the mechanisms remain unclear. A pooled analysis of observational studies showed a 30 % reduction in the incidence of colon carcinoma and colorectal polyps among current HT users. The results of RCTs are inconsistent. The HERS II trial showed a nonsignificant protective effect of estrogen use (RR 0.81, 95 % Cl 0.46-1.45). The WHI demonstrated no significant difference in rates of colorectal cancer (HR 1.08, CI 0.75-1.55) or total cancer among users of estrogen vs placebo (HR 0.93, CI 0.81-1.07) (The Women's Health Initiative Steering, Committee 2004), but the combination of estrogen plus progestin decreased the risk of colorectal cancer (HR 0.63, CI 0.43-0.92) and total cancer (HR 0.76, CI 0.69-0.85) (Rossouw *et al* 2002).

Recent studies have revealed conflicting results concerning ovarian cancer risk, with no change (Sit *et al* 2002), or a tendency towards an increased risk among ever users of HT compared with never users, with the risk increasing further with long-term use after ten years (from 1.2-1.6 to 1.8-2.2) (Riman *et al* 2004). In a randomized comparative trial, HR associated with invasive ovarian cancer in connection with continuous combined HT (0.625mg conjugated equine estrogen (CEE) plus MPA) was 1.58 (95 % CI 0.77-3.24) (Anderson *et al* 2004). There is less information about the effects of estrogen and progestin components of HT separately on ovarian cancer risk, but continuous combined HT in short-term use may not be detrimental (Riman *et al* 2004). The mechanisms, by which HT increases the risk of ovarian cancer, remains obscure (Riman *et al* 2004).

3.7. Effects in early vs. late postmenopausal women

Older women differ from younger women in their reasons for starting and stopping HT. While osteoporosis has been the predominant reason for older women to begin HT, the relief of vasomotor symptoms is the major reason for younger women. However, 10-15% of 70-year-old women suffer from vasomotor symptoms. Early discontinuation of HT (62 %) is common and more frequent among older (more than 65 years) than younger (50-55 years) women. Intolerance to treatment, particularly vaginal bleedings and breast tenderness, is the predominant reason for stopping HT (Ettinger *et al* 1999).

At the first few postmenopausal years bone turnover rate increases and then goes down before increasing again in late senescence (Mazess 1982, Garnero *et al* 1994). Low estradiol levels are correlated to decreased cortical and trabecular BMD in late postmenopause (Khosla *et al* 2005). It was demonstrated recently that the lower the endogenous estradiol level, the higher is the response of BMD to HT in elderly women (Rapuri *et al* 2004). In women over 65 years of age, continuous combined low-dose estrogen (1 mg E2V) (Heikkinen *et al* 2000) or 0.3 mg CEE per day (Recker *et al* 1999) with MPA for 4 years resulted in mean BMD increases of 6.2% and 3.5 % at the spine, and 2.9% and 0.3 % at the femoral neck, respectively (Heikkinen *et al* 2000). These treatments were well tolerated by most of the women.

Even an ultra-low-dose of 0.25 mg of oral 17β-estradiol per day significantly increased BMD in elderly women (>65 years old) at the hip (+2.6%), the spine (+2.8%), and in the total body (+1.2%), and reduced bone turnover, with minimal adverse effects (Prestwood *et al* 2003). In postmenopausal women (mean age 53 years) transdermal E2 at doses of 0.025, 0.05, 0.06, and 0.1 mg/day increased lumbar spine BMD by 2.37%, 4.09%, 3.28%, and 4.70%, respectively, and total hip BMD by 0.26%, 2.85%, 3.05%, and 2.03%, respectively. The increase of BMD may be dependent on the dose of estrogen and the addition of progestin (Weiss *et al* 1999,

Greenwald *et al* 2005). The effect of ultra-low-dose estrogen on BMD is seen in early and late postmenopausal women, but they have not been compared in the same study. In early menopause the intensity of symptoms usually determines the dose of estrogen. There are still no data on the effects of low-dose estrogen on fracture risk.

Less than 1 % of the US noninstitutionalized population under 45 years of age is edentulous, but this proportion increases to 40 % for those of 65 years and older. The proportion of women with edentia decreases with increasing duration of HT and denture wearing is also less common in estrogen users vs. non-users (Paganini-Hill 1995, Grodstein *et al* 1998).

HT relieves menopausal symptoms (McNagny 1999, MacLennan *et al* 2001) and improves the general well-being of early postmenopausal women (Wiklund *et al* 1992, Daly *et al* 1993, Zethraeus *et al* 1997, Genazzani *et al* 2002, Sintonen *et al* 2003). In elderly women HT seems to have mixed effects. In the WHI study (mean age of the women 63 years) HT was associated with a statistically significant, but not clinically meaningful QoL benefit. HT decreased pain and sleep disturbances, and improved physical functioning (RAND 36) (Hays *et al* 2003). In the HERS study (mean age of the women 67 years) HT seemed to have only a limited effect on HRQoL in women without vasomotor symptoms. However, women with vasomotor symptoms had less depression and improved in emotional measures with HT (Hlatky *et al* 2002).

In the HERS study the risk of VTEs was increased by a factor of 2.7 among elderly women assigned to receive estrogen-progestin therapy (Manson and Martin 2001), which is similar to the risk of VTEs in meta-analysis of 12 studies (RR 2.14, CI 1.64-2.81 overall, and RR 3.49, CI 2.33-5.59 during the first year) (Nelson *et al* 2002). In the WHI with CEE and MPA the risk of venous thrombosis was 3.5 per 1000 person-years for women taking estrogen plus progestin and 1.7 per 1000 person-years for women taking placebo (RR 2.06; 95% CI, 1.57-2.70). Compared with women on placebo and aged 50 to 59 years, the risk ratio of VTE when on HT increased with age. The RR was 4.28 (95% CI, 2.38-7.72) for women aged 60 to 69 years and 7.46 (95% CI, 4.32-14.38) for women aged 70 to 79 years (Cushman *et al* 2004).

4. Bisphosphonates

4.1. General principles

Bisphosphonates are synthetic analogs of the naturally occurring pyrophosphate. They are widely used in the treatment of osteoporosis, malignant hypercalcemia, myeloma and bone metastases. Bisphosphonates can be divided into two groups with different molecular structure and mechanisms of action.

The nitrogen-containing bisphosphonates (alendronate, pamidronate, risedronate, zoledronate and ibandronate) inhibit osteoclast-mediated bone resorption through the inhibition of farnesyl diphosphate synthase in the mevalonate pathway (Fisher *et al* 2000). This results in impaired protein prenylation and may affect the function of small GTPases in osteoclasts. These proteins are important regulators of vesicle transport in cells. Alendronate inactivates osteoclasts by mechanisms that impair their intracellular vesicle transport resulting in the accumulation of unprenylated, non-functional proteins, and in consequent apoptosis (Manolagas 2000, Alakangas *et al* 2002).

Pyrophosphate-resembling bisphosphonates (clodronate and etidronate) are metabolized in cells into ATP-like analogs, which mediate cellular effects, such as the induction of apoptosis.

Adverse effects of bisphosphonates are gastritis, esophagitis and gastric ulcers, but these occur in less than 1 % of patients (Devogelaer 1998, Stevenson *et al* 2005).

4.2. Effects on bone

In bone tissue bisphosphonates inhibit osteoclasts and prevent the apoptosis of osteocytes.

Alendronate reduces postmenopausal bone loss (Hosking *et al* 1998, Cranney *et al* 2002), and significantly improves lumbar spine and hip BMD in osteoporotic women (Liberman *et al* 1995, Black *et al* 1996, Pols *et al* 1999, Tonino *et al* 2000, Cranney *et al* 2002). In postmenopausal women with a previous vertebral fracture (age 55-81 years) alendronate approximately halved the risk of vertebral and forearm fractures, and also that of hip fracture (Liberman *et al* 1995, Black *et al* 1996, Karpf *et al* 1997, Black *et al* 2000, Cranney *et al* 2002).

Alendronate does not impair bone mineralization at the doses that maximally inhibit bone resorption (Rodan *et al* 1993). It is equally effective and well tolerated in osteoporotic women aged more or less than 70 years. The response in BMD is dose-dependent, with even at daily dose of 5.0 mg producing favorable effects (Bone *et al* 1997).

4.3. Effects on cardiovascular risks and surrogate markers

Bisphosphonates accumulate in artery walls and cause vasodilatation, and theoretically they may reduce the risk of CVD (Ylitalo *et al* 1998). They also cause an acute phase response and increase TNF-α, IL-6 (Thiebaud *et al* 1997) and Lp(a) levels and may cause a febrile reaction, when administereed intravenously (Lippi *et al* 1998). Bisphosphonates affect the cells of the immune system. The nitrogen-containing bisphosphonates are considered pro-inflammatory, but pyrophosphate-resembling bisphosphonates are considered to be anti-inflammatory and have shown a

promising anti-atherosclerotic potential (Hewitt *et al* 2005). However, there is no clinical evidence of the effects of bisphosphonates on the cardiovascular events.

4.4. Effects on oral health

Bisphosphonates can inhibit the catalytic activity of MMPs in vitro, and this might be one potential mechanism behind the down-regulation of bone resorption (Teronen *et al* 1999). MMPs represent the family of tissue-degradative host proteinases, and they are involved not only in pathologic tissue destruction but also in tissue remodelling associated with tooth development and wound healing (Birkedal-Hansen 1993, Salo *et al* 1994, Pirilä *et al* 2001).

Bisphosphonates might also have a role in adjunct therapy for periodontal disease (Jeffcoat 1998, El-Shinnawi and El-Tantawy 2003, Rocha *et al* 2004). For this purpose the ability of bisphosphonates to act as MMP inhibitors could be useful (Golub *et al* 1997). Bisphosphonate treatment improves the clinical outcome of non-surgical periodontal therapy of chronic periodontitis (Lane *et al* 2005) and may also result in the promotion of bone formation around endosseous implants (Tenenbaum *et al* 2002). The decreased salivary flow rate in the menopausal period has been shown to increase ($p=0.03$) after combination treatment of HT with alendronate and calcium in osteoporotic women (mean age 50.7 years) (Yalcin *et al* 2005).

Injectable regimens of bisphosphonates, pamidronate and zoledronate, may cause osteonecrosis of the jaws, which is mostly observed in patients treated for malignant diseases, but 7 of 63 cases were associated with oral bisphosphonates (Hellstein and Marek 2004, Hellstein and Marek 2005).

4.5. Effects on quality of life

Bisphosphonates are bone-tissue specific, with very few side effects and no risk of carcinogenesis. Women with severe osteoporosis respond to bisphosphonates with increasing BMD, with the future fracture risk being reduced by 40-50%. This results in decreased pain, and better mobilization, social well-being and QoL (Devogelaer 1998, Dursun *et al* 2001). The risk of hospitalization, morbidity and mortality associated with hip fractures is also reduced (Seeman 1997, O'Connell 1999, Epstein 2000).

5. The combination of hormone therapy and bisphosphonates

Women with severe osteoporosis and those who have failed to respond optimally to estrogen or bisphosphonate alone might benefit when they combine these two antiresorptive agents, with different mechanisms of action. However, previous studies have given conflicting results concerning whether the combination produces a net gain in BMD over either drug alone (Wimalawansa 1995, Wimalawansa 1998,

Lindsay *et al* 1999, Bone *et al* 2000, Tiras *et al* 2000, Palomba *et al* 2002, Greenspan *et al* 2003). There are no data on fracture risk concerning combination therapy with estrogen and bisphosphonates. Combination therapy with estrogen and bisphosphonate has been studied mostly in early postmenopausal women (Table 2).

6. Calcium and vitamin D

Inadequate dietary intake of calcium and vitamin D may contribute to the high prevalence of osteoporosis among older persons. Deficiency of vitamin D may lead to secondary hyperparathyroidism, and alone or combined with the deficiency of calcium, may increase bone loss in elderly women (Chapuy *et al* 1992, Ooms *et al* 1995, Dawson-Hughes *et al* 1997). Vitamin D supplementation has been recommended especially for elderly and institutionalized people, but the net gain in BMD after supplementation has been moderate (1-2%) at the femur (Chapuy *et al* 1992, Ooms *et al* 1995) and spine (Dawson-Hughes 1997). In treatment of postmenopausal women aged more than 60 years, an additive effect was found when low-dose hormone therapy (0.31mg CEE and 2.5mg MPA) was combined with a 1μg daily dose of 1alpha-OH-vitamin D3 (alphacalcidol) with the lumbar spine BMD increasing by 8.75 % vs. 2.32% on HT alone in 2 years (Mizunuma *et al* 2006).

Vitamin D supplementation decreases vertebral fractures (RR 0.63, 95% CI 0.45-0.88) and tends to decrease non-vertebral fractures (RR 0.77, 95% CI 0.57-1.04) (Papadimitropoulos *et al* 2002). A daily dose of 700 to 800 IU of vitamin D has been shown to reduce the risk of hip fractures by 26 % and also to decrease fall injuries, whereas a dose of 400 IU daily has been inefficient in the prevention of fractures (Bischoff-Ferrari *et al* 2003, Bischoff-Ferrari *et al* 2005).

Calcium supplementation alone has a small positive effect on BMD and a non-significant reductive effect on the incidence of vertebral fractures (Shea *et al* 2002). It has been shown to prevent new vertebral fractures in calcium-deficient osteoporotic women with earlier vertebral fractures, but not in those without previous fractures (Recker *et al* 1996).

Table 2. Combination treatments of hormone therapy and bisphosphonates
L: lumbar, F: Femur, Fn: Femur neck, Ttr: trochanter, Rad: Radius, Al: alendronate, CEE: conjugated estrogen, E2: estradiol, P: progesterone

Study	N	Duratio months	Dosage	Population Study design	Age mean	BMD change from baseline **Combination** vs HT vs Al	Significant P<0.05
Wimalawansa et al 1995	58	48	**2.5g 17β-E2 transderm +200mgP + 400mg etidonate (1)** vs 2.5g 17β-E2 transderm +200mg P (2) vs 400mg etidronate 14d (3) vs Ca (4)	Postmenopausal + 1000mg Ca normal BMD	52.6	**L**: **10.9** % **(1)** Vs 6.8 % (2) vs 6.8 % (3) vs -3.8 % (4) **F**: **7.3** % **(1)** vs 4.0 % (2) vs 1.2 % (3) vs -5.0 % (4)	**1 vs. 2,3,4** **1 vs. 2,3,4**
Lindsay et al 1999	428	12	**HT +MPA ongoing** **+ 10mg Al (1)** vs+placebo (2)	Osteoporosis +1000mg Ca +400IU D	62	**L**: **3.6** % **(1)** vs 1%, (2) **Ftr**:**2.7**% **(1)** vs 0.5%,(2) **Fn**:**1.7**% (1) vs 0.8 (2)	**1 vs. 2** **1 vs. 2**
Bone et al 2000	425	24	**0.625mg CEE+10mg Al (1)** vs 0.625mg CEE (2) vs 10mg Al (3)	Osteoporosis or osteopenia hysterectomized, +Ca 500mg	62	**L**: **8.3**% **(1)** vs 6.0% (2) vs 6.0%(3) **F**: **4.7**% **(1)** vs 3.4 % (2) vs 4.0 % (3)	**1 vs. 2,3** **1 vs. 2,3**

Tiras et al 2000	120	12	**2mg E2+1mg NETA+10mg AI (1)** vs 2mg E2+1mg NETA (2) vs 10mg AI (3)	Osteoporosis BMD<2SD +Ca 1500mg	52	**L**: **8.4** % **(1)** vs 2.6 % (2) vs 7.2 % (3) **Fn**: **4.6** % **(1)** vs 3.2 % (2) vs 3.0% (3)	
Harris et al 2001	542	12	**0.625mg CEE+5mg MPA + 5mg risedronate (1)** vs 0.625mg CEE +5mg MPA (2)	Postmenopausal +1000mg Ca (+500 IU D) normal BMD or osteopenia	58	**L**: **5.2** % **(1)** vs 4.6 % (2) **Fn**: **2.7** % **(1)** vs 1.8 % (2) **Ftr**: **3.7** % **(1)** vs 3.2 % (2) **Rad**: **0.7** % **(1)** vs 0.4 % (2)	**1 vs. 2** **1 vs. 2**
Palomba et al 2002	150	24	**2mg E2+ 10mg AI (1)** vs **2mg E2+ 5mg AI** (2) vs 2mg E2 + placebo (3)	Osteoporosis Hysterectomized surgically postmenopausal	60-62	**L: 8**% **(1)** vs **8**% **(2)** vs 4% (3) **F**: **6**% **(1)** vs **6** % **(2)** vs 2% (3)	**1,2 vs. 3** **1,2 vs. 3**
Greenspan et al 2003	373	36	**0.625mg CEE+5mg MPA +10mg AI (1)** vs 0.625mg CEE + 5mg MPA (2) vs 10 mg AI (3)	Osteoporosis or osteopenia +1000mg Ca +400-800 IU D	71	**L**: **10.4** % **(1)** vs 7.1 % (2) vs 7.7 % (3) **F**: **5.9** % **(1)** vs 3.0 % (2) vs 4.2 % (3)	**1 vs. 2,3** **1 vs. 2,3**

Aims of the study

The main purpose of the present study was to explore the effects of hormone therapy in elderly women between 65 and 80 years of age. Two cohorts of women were studied

I. In a cohort of women with osteoporosis, HT was compared with alendronate with respect to their effects on

1. Bone mineral density and markers of bone turnover

2. Surrogate markers of cardiovascular diseases, C-reactive protein and E-selectin

3. Oral health parameters, with special emphasis on periodontal disease, symptoms, saliva, and gingival crevicular fluid matrix metalloproteinase-8 levels

II. In a population-based cohort the effect of hormone therapy on health-related quality of life was examined using a standardized questionnaire (15 D).

Subjects and Methods

1. Subjects

1.1. The cohort of postmenopausal women with osteoporosis (Studies I-III)

We enrolled 90 postmenopausal women, 65–80 (mean 71) years of age, into this double-blind, randomized, 2-year study. Inclusion criteria were BMD at the lumbar spine (n=50) or femoral neck (n=71) at least 2.5 SD below the mean of a reference population of young premenopausal women. Exclusion criteria included metabolic bone disease other than postmenopausal osteoporosis, general contraindications of HT, use of bone-active agents (any previous use of bisphosphonates, concomitant use of oral glucocorticoids, or HT use less than 6 months before the study), diseases that affect bone turnover, history of gastrointestinal mucosal disorders (erosive gastritis, gastric ulcer or esophagitis), history of a prior thromboembolic disease, liver or kidney disease, insulin-treated diabetes, history of uterine or breast cancer, or uncontrolled hypertension. Hypercholesterolemia (13 patients, 9 using statins), cardiovascular diseases (23 patients), hypothyroidism requiring thyroxine treatment, with thyroid stimulating hormone levels within the normal range (11), and asthma (11) were not reasons for exclusion from the trial. The women were randomized to one of three treatment regimens: continuous combined HT (2 mg estradiol plus 1 mg norethisterone acetate (NETA) orally; Kliogest®; Novo Nordisk, Copenhagen, Denmark, n=30), alendronate (10 mg Fosamax®; Merck & Co. inc., NJ, USA, n=30), or HT plus alendronate (n=30). The baseline characteristics of the study population according to subsequent treatment are described in Table 3.

Out of these 90, a subgroup of 60 women (20 in the HT group, 18 in the alendronate group, 22 in the combination group) were willing to participate in a dental examination (Study III), but only 40 subjects (44%) came to the dental examination two years later. The main reason for refusal was lack of interest. The drop-outs were evenly distributed among the study groups. In the loss analysis the only difference was found to be the worse subjective feeling of general health in the drop-out group, but no other differenceses in health parameters existed between the groups. Exclusion criteria for MMP-8 sampling were gingival bleeding or an edentulous state.

The Ethics Committee of the Department of Obstetrics and Gynecology, Helsinki University Central Hospital, approved the study protocol and all subjects gave written informed consent.

1.2 The population-based cohort of elderly postmenopausal women (Study IV)

The subjects were participants in an ongoing intervention study, which is aimed at elucidating the effectiveness of an educational program in the prevention of osteoporosis and fractures. A random sample of the female population in Southern Finland (Uusimaa region) within the age group of 60-70 years was drawn from the population registry, which covers all subjects living in Finland. In all 4200 women were invited to take part in the trial; 2181 (52 %) accepted the invitation and were recruited between 1996 and 2000. They were randomized either to the educational program or to the control group.

In 2002 all 2181 participants were invited by letter to take part in the present study designed to illuminate health-related quality of life in the postmenopausal period. The participants were asked to fill in a quality of life questionnaire and to answer to additional questions. Overall, 1663 women (76 %; 835 participants in the educational program, 828 controls) aged 65-80 years completed the questionnaire; 585 were HT users (mean age of 67.5 years) and 1078 were non-users (mean age of 68.9 years).

The Ethics Committee of the Department of Medicine, Helsinki University Central Hospital approved the study protocol and all subjects gave written informed consent. (Table 4)

Table 3. Characteristics [mean (SD) or n (%) or medians (range)] of the study population (n=90).

	Alendronate n=30	**HT n=30**	**Combination n=30**	**p**
Age (yr)	70 (68–74)	70 (69–73)	72.5 (68–74)	0.45
History of hysterectomy	10 (33%)	10 (33%)	10 (33%)	
Years since Menopause	22.6 (6.5)	21.8 (4.9)	23 (4.7)	0.69
Smokers (n)	5	1	5	0.19
Dietary calcium (mg/d)	817 (434)	707 (321)	687 (287)	0.16
Alcohol (doses/w)	0.7 (1.3)	1.2 (2.7)	1.7 (3.1)	0.21
Height (cm)	158.8 (5.2)	158.2 (5.8)	160.7 (4.4)	0.15
Weight (kg)	63.5 (55.0–70.3)	65.0 (59.8–70.3)	61.5 (54.8–74)	0.94
BMI	25 (22–27)	25 (24–28)	23 (22–28)	0.45
BPsyst (mmHg)	150 (140–166)	151 (138–171)	150 (140–163)	0.74
BPdiast (mmHg)	90 (79–95)	90 (84–97)	90 (80–97)	0.27
BMD Lumbar spine (g/cm^2)	0.740 (0.689–0.802)	0.741 (0.697–0.860)	0.771 (0.722–0.833)	0.44
BMD Femoral neck (g/cm^2)	0.609 (0.555–0.649)	0.624 (0.603–0.651)	0.614 (0.563–0.655)	0.51
BMD Trochanter (g/cm^2)	0.541 (0.088)	0.579 (0.073)	0.565 (0.084)	0.21
BMD Total Hip (g/cm^2)	0.733 (0.113)	0.778 (0.085)	0.746 (0.084)	0.13
Urinary NTX (nmol/mmol Crea)	71.5 (51.7–89.7)	66.9 (48.2–90.6)	67.1 (52.1–83.7)	0.92
Serum PINP (µg/l)	49.5 (38.8–66.8)	50.0 (34.5–58.8)	47.0 (36.8–59.3)	0.76
Serum 25(OH)D (nmol/l)	46.0 (37.8–66.0)	52.0 (38.8–65.0)	54.5 (28.0–66.3)	0.97
CRP (mg/l)	1.7 (1.4)	1.4 (1.2)	1.4 (1.8)	0.93
E-Selectin (µg/l)	42.6 (17.1)	47.4 (17.1)	46.3 (16.8)	0.11
SHBG (nmol/l)	89.9 (27.6)	78.8 (31.6)	106.3(32.9)	0.34

Table 4. Characteristics [mean (SD) or n (%)] of the population-based cohort according to HT use (study IV)

	HT non-users n=1078	**HT users n=585**	**p**
Age, years	68.9 (3.4)	67.5 (3.1)	<0.0001
Flushing			
All of the time			
Most of the time	8 (1.0)	1 (0.2)	0.001
Some of the time	18 (2.0)	6 (1.0)	
Little of the time	52 (5.0)	17 (3.0)	
None of the time	174 (16)	62 (10.8)	
	807 (76)	490 (85)	
Number of chronic diseases			
0	257 (26)	172 (32)	0.068
1-3	688 (70)	350 (64)	
4 or more	41 (4)	23 (4)	
Number of medication			
0	226 (23)	139 (25)	0.009
1-2	347 (35)	223 (41)	
3 or more	417 (42)	188 (34)	
Number of antidepressant			
users	885 (89.5)	481 (87)	0.37
Never	32 (3.4)	19 (3)	
More than 5 years ago	63 (7.1)	50 (10)	
Now or during 5 years previously			
Basic education			
Primary school	485 (51)	193 (36)	<0.0001
Comprehensive school	298 (32)	173 (33)	
High school	160 (17)	165 (31)	
Professional education			
No	147 (17)	59 (12)	<0.0001
Vocational courses	345 (40)	173 (34)	
Vocational school	124 (14)	65 (13)	
College	155 (18)	109 (21)	
University	101 (11)	104 (20)	

2. Study protocols:

2.1. Effect of hormone therapy, alendronate, and their combination on bone mass and turnover (Study I)

The women were randomized (using an outside computer program) to one of three treatment regimens: continuous combined HT (2 mg estradiol plus 1 mg NETA orally; Kliogest®; Novo Nordisk, Copenhagen, Denmark, n=30), alendronate (10 mg Fosamax®; Merck & Co. Inc., NJ, USA, n=30), or HT plus alendronate (n=30). The principle of double dummy techniques was followed so that each regimen was similar in appearance. The women were instructed to take alendronate or its placebo in the morning, at least 30 min before the first meal of the day, with a glass of water, and to remain upright for at least 30 min after dosing. HT or its placebo was taken in the evening. At baseline, dietary calcium intake was assessed by using a questionnaire. In addition to the study medication, the participants were instructed to take calcium supplementation (500–1000 mg/day) and vitamin D (400 IU/day) during the fall and winter months from October to April, but we did not provide them. Compliance in use of the study medication was assessed by counting the unused tablets. The subjects were seen at baseline and at 6, 12, 18 and 24 months. At each visit, a clinical and a pelvic examination were performed. The BMD of the lumbar spine and hip (femoral neck, trochanter and total hip) was measured at baseline and at 12 and 24 months using the same densitometer over the whole duration of the study. Serum and second void urine samples were obtained in the morning after an overnight fast at baseline and at 6, 12, 18 and 24 months for assay of biochemical markers of bone turnover, and serum 25-hydroxyvitamin D (S-25(OH)D). The samples were stored at –28 ºC until assayed.

2.2. Effect of hormone therapy, alendronate, and their combination on surrogate markers of cardiovascular diseases (Study II)

This study was performed in the same study population as in Study I by collecting blood samples for the determination of serum CRP, E-selectin, and SHBG at baseline and at 6 and 12 months.

2.3. Effect of hormone therapy, alendronate, and their combination on oral health (Study III)

Dental, periodontal and intra- and extraoral status was recorded at the beginning and at the end of the study at 24 months. For the saliva and gingival crevicular fluid (GCF) tests the subjects were instructed not to eat or smoke for two hours prior to the examination. Panoramic tomography of the jaws (OPTG) was carried out before the clinical examinations. A structured questionnaire was given to all the subjects prior to the examinations. The questionnaire comprised multiple-choice questions on smoking, self-assessed general and dental health, and an enquiry regarding the last visit to a dentist. Subjective concepts of periodontal health of the women were investigated by means of multiple-choice questions on gingival bleeding during tooth brushing. Resting and stimulated flow rates of saliva were measured. Resting saliva was collected by means of the free flowing method for 3 minutes (Meurman and Rantonen1994, Närhi1994). To stimulate saliva secretion a 1-gram piece of paraffin wax was given to the women for them to chew. The collection time for stimulated saliva was also 3 min. All the dental examinations were carried out by the same examiner (L.T.). Samples of GCF for assay of MMP-8 levels were collected from periodontal pockets of two teeth of each subject (n=34). Exclusion criteria for MMP-8 sampling were gingival bleeding or an edentulous state (11 were excluded). Before taking the sample for MMP-8 measurement supra-gingival plaques were removed and the sampling sites were isolated with cotton rolls and dried gently to avoid saliva contamination. The samples of GCF were taken with a filter-paper sampling strip. The strip was placed into the gingival crevice for 30 seconds. It was then placed in a test tube and frozen at -20°C until analysed (Sorsa *et al* 1999, Mäntylä *et al* 2003).

2.4. Effect of hormone therapy on health-related quality of life in the population-based cohort of elderly postmenopausal women (Study IV)

The participants were asked to fill in a quality of life questionnaire (generic 15 D) and to answer additional questions. All participants were asked if they currently used HT. Flushing symptoms were enquired about by the question “During the last week, did hot flushes bother you?”, and information was classified as “all of the time”, “most of the time”, “some of the time”, “a little” and “none of the time”. We also obtained information on basic education (primary, comprehensive or high school), professional education (none, vocational courses, vocational school, college or university), the number of ongoing chronic diseases, and continuous medication. Medication for depression was of particular interest to us.

3. Measurements

3.1. Bone mineral density

The BMD of the lumbar spine (L1-4) and hip (femoral neck, trochanter and total hip) was measured at baseline and at 12 and 24 months by dual-energy X-ray absorptiometry (DXA) (Hologic, Inc., QDR 1000W, Waltham, MA, USA), using the same densitometer over the whole duration of the study. Data were given as areal density (g/cm²). The coefficient of variation (CVs) of the spine and femoral neck measurements were 0.9 % and 1.2 %, respectively.

Primarily the BMD of L1-4 was used for the analyses, but in cases of severe changes (arthrosis, fracture, scoliosis, etc) L1-3, L1-2, L2-3 or L 3-4 were used instead. In follow-up, the BMD of the same vertebrae was always analyzed.

3.2. Bone turnover markers and serum 25(OH)D

The urinary N-telopeptide (NTX) of type I collagen as related to creatinine was measured as a marker of bone resorption, using an ELISA (Osteomark NTx Test, Ostex International, Seattle, WA, USA). The intra-assay CV was 8 % and the interassay CV 13 %. The serum aminoterminal propeptide of human type I procollagen (PINP) was measured as a marker of bone formation, using RIA kits (Orion Diagnostica, Espoo, Finland). The intra- and interassay CVs were 7 %. Serum 25(OH)D was measured by RIA (Immunodiagnostic Systems Ltd (IDS), Boldon, UK). The intra- and interassay CVs were 6 %.

3.3. C-reactive protein, E-selectin, and sex hormone binding globulin

CRP was measured immunochemically using of monoclonal antibodies produced in mice (DADE-Behring Marburg GmbH, Marburg, Germany). The intra- and inter-assay CVs were 2 % and 3 %. Because CRP concentrations rise in acute infections, seven subjects with CRP values exceeding 10 mg/L were excluded.

E-selectin was measured by a sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN). The intra-assay variation CV was below 5 %, and the interassay CV below 9 %.

SHBG was measured with a solid phase, two-site fluoroimmunometric assay (DELFIA®, Wallac Oy, Turku, Finland). The intra-assay CV was less than 2 %, and the interassay CV 5 %.

3.4. Oral health measurements and matrix metalloproteinase-8

3.4.1. Clinical dental recordings and questionnaire

Dental status was recorded using WHO criteria (Anonymous 1987). Because not all women remembered their history of dental treatment, especially reasons for tooth

extractions, the third molars were included in recording the numbers of decayed teeth (DT), filled teeth (FT) and the total number of teeth. However, in calculating the WHO DMFT-index the third molars were not included (M=missing teeth). A review of OPTG X-ray films by a radiologist specialized in oral disease, was available at the clinical examination. The number of incidences of periapical radiolucency, endodontically treated teeth, and furcation lesions of multi-rooted teeth were counted from the X-ray films.

3.4.2. Periodontal health status

Periodontal health status and need of treatment were recorded using the WHO Community Index of Periodontal Treatment Need (CPI) (Ainamo *et al* 1982). All surfaces of the teeth were examined. The mouth sextants were scored on the basis of the worst finding in the respective sextant. Periodontal probing depths were measured to the nearest mm from the gingival margin to the bottom of the periodontal pocket at four surfaces of each tooth with a WHO periodontal ball-point probe (tip diameter 0.5 mm).

3.4.3. Buffering capacity

Buffering capacity was assessed immediately after collecting saliva using the Dentobuff Strip® method (Orion Diagnostica Ltd., Espoo, Finland). Score 1 corresponds to buffering end-pH > 6, score 2 to pH 4.5-5.5 and score 3 to end-pH less than 4.5. A pH value < 4.5 (score 3) was recorded as a low buffering capacity (Närhi *et al* 1993).

3.4.4. Oral yeasts

To assess the prevalence of *yeasts*, the Dentocult CA® dip slide method (Orion Diagnostica Ltd, Espoo, Finland) was used. Score 1 corresponds to 0 to 20 colony-forming units (CFU) per ml, score 2 corresponds 21-50 CFU/ml, and score 3 to more than 50 CFU/ml.

3.4.5. Salivary total protein and albumin

The salivary concentrations of protein and albumin were determined by colorimetric analyses. Salivary immunoglobulin concentrations (IgG, IgM, IgA) were analyzed by means of enzyme immunoassays.

3.4.6. Resting and stimulated flow rates of saliva

Resting flow rates of saliva below 0.1 ml/min and stimulated flow rates below 0.7 ml/min were regarded as reduced salivary flow rates (Meurman and Rantonen 1994, Närhi 1994).

3.4.7. Matrix metalloproteinase-8

Levels of MMP-8 in the GCF samples were determined by a time-resolved immunofluoresence assay (IFMA). Fluorescence was measured using a 1234 Delfia Research Fluorometer (Wallac, Turku, Finland)(Hanemaaijer et al 1997, Liede et al 1999, Sorsa et al 1999). Specific monoclonal antibodies 8708 and 8706 to MMP-8 were used as catching and tracer antibodies, and the tracer antibody was labelled

using europium-chelate. The specificities of the monoclonal antibodies against MMP-8 corresponded to those of polyclonal MMP-8 antibodies and they did not detect other MMPs (Lauhio *et al* 1994, Hanemaaijer *et al* 1997, Liede *et al* 1999, Sorsa *et al* 1999). The results were reported as total MMP-8 (μg/ml) in the sample.

3.5. Health-related quality of life questionnaire (15D)

Health-related quality of life measurement

The 15D questionnaire is a generic, comprehensive, 15-dimensional, standardized, self-administered questionnaire giving a measure of HRQoL that can be used both as a profile and single index score measure. The dimensions are: mobility, vision, hearing, breathing, sleeping, eating, speech, elimination, usual activities, mental function, discomfort and symptoms, depression, distress, vitality and sexual activity. Each dimension is divided into five ordinal levels, by means of which more or less of the attribute can be distinguished. The person ticks, for each dimension, the level that best describes his or her health status. The single index (15D score) on a 0-1 scale, representing the overall HRQoL, is calculated from the health state descriptive system by using a set of population-based preference or utility weights. Such a weight for each level of each dimension is obtained by multiplying the level value by the importance weight of the dimension at that level. The level values on a 0-1 scale, reflecting the goodness of the levels relative to no problems on the dimension (=1) and to being dead (=0), and the importance weights summing up to unity, have been elicited earlier from representative population samples. A difference of > |0.03| in the overall 15D score is clinically important in the sense that on average people can feel the difference (Sintonen1994, Stavem 1999, Hawthorne *et al* 2001, Sintonen 2001, Sintonen *et al* 2003).

4. Statistical analyses

In Study I data with normal distributions are expressed as means with SDs; otherwise as medians with interquartile ranges (IQRs). In comparisons between the study groups, normally distributed variables were studied using one-way ANOVA followed by Bonferroni correction in paired comparisons. Those data non-normally distributed were tested by means of Kruskal-Wallis one-way ANOVA on ranks. Bone mineral density changes were analyzed by repeated measures repeated measures (RM) ANOVA or by using percentage changes from 0 to 12- and 24-month time points. Because the assumptions for repeated measures ANOVA were not fulfilled for the biochemical results, a summary variable “area under the curve” (AUC) was calculated and analyzed by ANOVA in the analysis of differences between the groups.

Additionally, Friedman RM ANOVA was used in the analysis of changes within a single study group. The analyses were carried out using NCSS 2000 software (NCSS Statistical Software, Kaysville, Utah, USA) or SigmaStat for Windows (Version 2.0, SPSS Inc., Chicago, IL, USA).

In Study II the SPSS 10.0 statistical package (SPSS Inc., Chicago, Illinois, United States) was used. The levels of the biomarkers at baseline or during the trial were compared with the Mann-Whitney U-test and the changes during the trial with the Sign test.

In Study III all the results were analyzed using SPSS for Windows version 11.0 software (SPSS Inc., Chicago, Illinois, United States) to detect differences between and changes within the study groups. Due to the small sample sizes only nonparametric analyses (Kruskal-Wallis test, Chi^2 test, Wilcoxon test) were used. Values of $p < 0.05$ were considered statistically significant.

In Study IV the difference in age of HT users vs. non-users was tested by using independent samples t-test. Differences between the groups in categorial variables (flushing, number of chronic diseases and medication, basic and professional education) were tested with the Chi-Square test.

Since the groups of estrogen users and non-users were not assigned randomly, any possible difference in the 15D score between the groups may be due to differences in important background and clinical characteristics between the groups. To account for such characteristics, the variance in the 15D score was explained by a Tobit regression model with age, the number of medication and chronic diseases as well as education as covariates in addition to a dummy indicating, whether the person was an estrogen user or not. The Tobit regression model is suitable for two reasons. The distribution of dependent variables (15D score) is not normal, but skewed and censored at 0 and 1 due to the fact that its range is 0-1 and the fact that a considerable number of observations were at the upper limit of 1 (7.5 %). The Tobit model accounts for these special features of the distribution.

Tobit models with the same explanatory variables were also used to create a 15D profile for both groups. The profiles indicate the average level value on a 0-1 scale for each group in each dimension, when the groups were standardized for age, number of medication and chronic diseases as well as education. A p-value of # 0.05 was regarded as statistically significant. The data were analyzed using Limdep 7.0 (Econometric Software, Inc., New York 1998) and SPSS version 13.0.

Results

1. Effect of hormone therapy, alendronate, and their combination on bone (Study I)

1.1. Bone mineral density

Lumbar spine BMD increased similarly in all treatment groups ($p<0.0001$ vs. baseline) (Fig. 2). The increases ranged from 6.8% to 8.4% at 12 months and from 9.1% to 11.2% at 24 months. Only HT increased femoral neck BMD statistically significantly at both 12 (+4.9%; $p<0.0001$) and 24 months (+5.8%; $p<0.0001$). At the latter time point, the HT group differed significantly from the other groups ($p<0.05$). The alendronate group exhibited a significant increase of +3.3% from baseline at 12 months ($p<0.05$), and the combination treatment group showed an increase of +2.7% at 24 months ($p<0.05$). All treatments raised trochanter BMD ($p<0.001$–0.0001 for differences from baseline at 12 months; $p<0.0001$ at 24 months), with the alendronate group showing the biggest increases both at 12 months (+5.8%; $p<0.01$ vs. the other groups) and 24 months (+8.5%; $p<0.01$ vs. the combination treatment group). Total hip BMD increased in all study groups ($p<0.05$–0.0001 for differences from baseline at 12 months; $p<0.0001$ at 24 months), with no significant differences between the treatments.

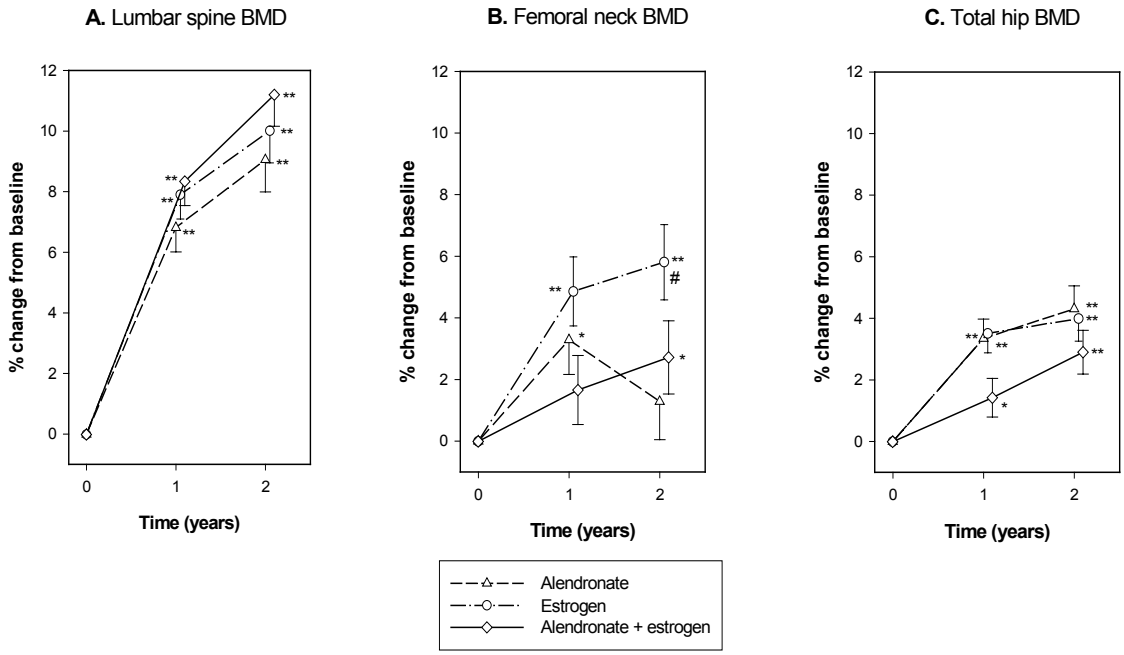


Fig. 2. Mean (SEM) percentage changes from baseline in bone mineral density (BMD) of lumbar spine (A), femoral neck (B), and total hip (C). *p<0.05, **p<0.0001 for differences from baseline. #p<0.05 for differences vs. the other two groups.

1.2. Biochemical markers of bone turnover

Significant reductions from baseline were seen in all treatment groups from 6 months onwards ($p<0.001$) in both urinary NTX and in serum PINP (Fig. 3). Percentage changes in urinary NTX ranged from -60.2% to -62.7% in the HT group, these being significantly smaller than those of -78.1% to -80.4% in the combination treatment group ($p<0.001$-0.0069). In the alendronate-only group the respective reductions ranged from -72.4% to -76.1%, which differed from those in the HT-only group at 24 months ($p=0.047$) and those in the combination group at 12 months ($p=0.002$). Serum PINP decreased less in the HT group (-53.6% to -59.8%) than in the other groups (-73.0% to -75.0% in the alendronate group [$p<0.001$ at 12 months]; -67.0% to -71.5% in the combination treatment group [$p<0.0001$ at 12 months; $p=0.013$ at 24 months]).

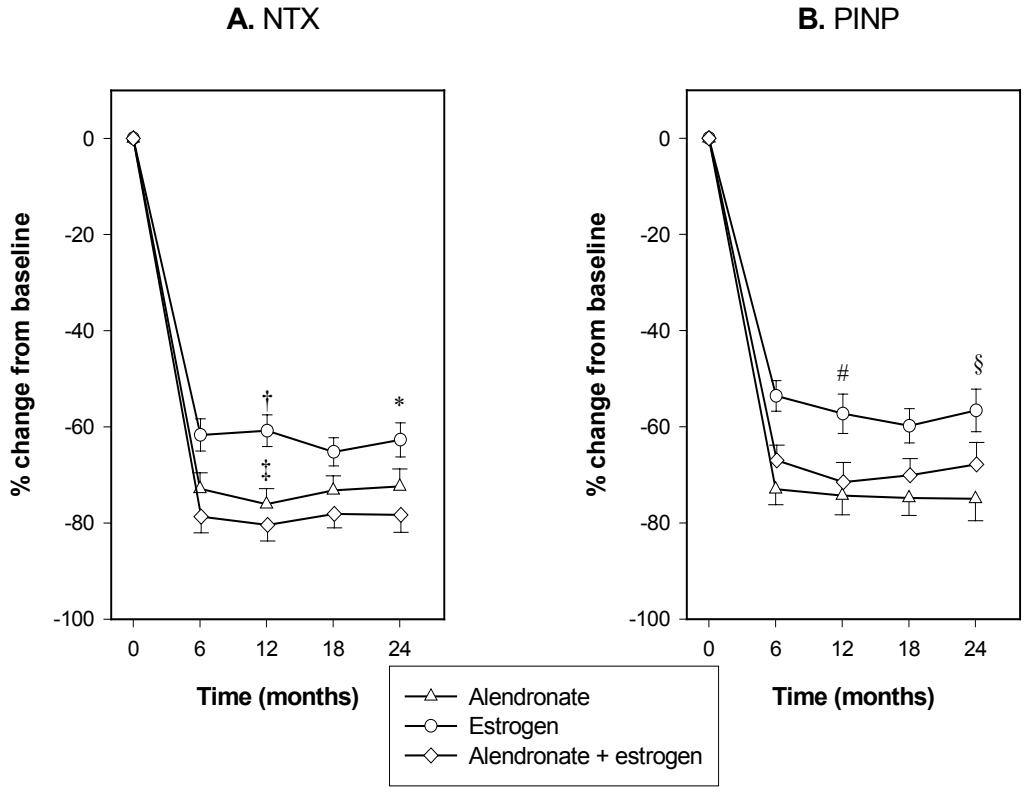


Fig. 3. Mean (SEM) percentage changes from baseline in bone turnover markers, urinary NTX (A) and serum PINP (B). Changes from baseline were significant in all three study groups ($p<0.001$, Friedman RM ANOVA). †$p<0.0001$ for the difference between the HT group and combination groups; ‡ $p=0.002$ for the difference between the alendronate and combination groups; # $p<0.001$ for the difference to the other two groups; * $p=0.047$ for the difference between the HT and alendronate groups and $p=0.0069$ for the difference between the HT and combination groups; § $p=0.013$ for the difference between the HT and combination groups.

1.3. Relationship between changes in bone mineral density and bone markers

In the whole study population, the maximum reduction in serum PINP levels correlated with the increase in lumbar spine BMD at both 12 (r=0.34, p=0.004) and 24 months (r=0.24, p=0.04). Respectively, the maximum drop from baseline in urinary NTX correlated with the increases in lumbar spine BMD at both time points (r=0.32-0.40, p<0.001-0.007) and in total hip BMD at 24 months (r=0.27, p=0.02). However, as calculated from these poor correlations (r^2 values) the marker changes explained only 10-15% of BMD changes.

1.4. Serum 25(OH)D

Serum 25(OH)D concentrations were similar in all treatment groups at baseline and they remained at the same level throughout the study. Hypovitaminosis D as defined as a serum 25(OH)D level less than 37 nmol/L (15 ng/mL) was found in 18.9%, 12.9%, and 17.2% of the whole number of the participants at baseline, 12, and 24 months, respectively.

2. Effect of hormone therapy, alendronate, and their combination on surrogate markers of cardiovascular diseases and serum sex hormone binding globulin (Study II)

2.1. C-reactive protein

In the HT group CRP concentrations were increased by a mean of 76.5% at 6 months (p < 0.001) and 47.1% at 12 months. Alendronate alone did not affect the levels of CRP and did not blunt the HT-induced rises in the HT + alendronate -group, in which the average rises were 50.0% at 6 months (p=0.031) and 52.9% at 12 months (p=0.019) (Table 5).

2.2. E-selectin

Concentrations of E-selectin in the HT group were decreased at 6 months by 24.3% (p<0.001) and at 12 months by 30.0% (p<0.001). Alendronate had no effect on E-selectin and did not block the effect of HT in reducing E-selectin levels (Table 5).

2.3. Sex hormone binding globulin

In the HT group concentrations of SHBG were increased at 6 months (14.8 %; p<0.05) and at 12 months (11.0%; p=0.09). Alendronate, or HT plus alendronate did not have any effect on SHBG levels. No significant correlation between the changes in CRP and SHBG emerged at the individual level (Table 5).

Table 5. Levels (mean ± SD) of CRP, E-selectin, and SHBG before and during treatment with estrogen-progestin (HT), alendronate, or HT+alendronate.

	Baseline	6months	12months
CRP (mg/L):			
HT	1.4 (1.2)	2.4 (2.1)**	2.0 (1.6)
Alendronate	1.7 (1.4)	1.5 (1.7)	1.7 (2.1)
HT+Alendronate	1.4 (1.8)	2.0 (1.6)*	2.1 (1.6)*
E-Selectin (µg/L):			
HT	47.4 (17.1)	35.9 (15.7)**	33.2 (14.6)**
Alendronate	42.6 (17.1)	43.8 (18.3)	40.7 (16.7)
HT+Alendronate	46.3 (16.8)	36.4 (15.2)**	35.9 (13.7)**
SHBG (nmol/L):			
HT	78.8 (31.6)	90.5 (29.2)*	87.5 (32.0)
Alendronate	89.9 (27.6)	86.5 (25.0)	87.7 (33.2)
HT+Alendronate	106.3(32.9)	96.5 (37.9)	91.8 (29.7)

*p<0.05 for changes from baseline

** p≤ 0.001 for changes from the baseline

3. Effect of hormone therapy, alendronate, and their combination on oral health (Study III)

3.1. Dental and oral status

No significant differences were observed in any dental or oral health status parameters between the groups at baseline. The number of patients with severe periodontitis increased (NS) in all the study groups in terms of both the CPI values and in the number of deep periodontal pockets. The increase in the number of deep periodontal pockets between baseline and follow-up recordings was significant in the HT and combination therapy groups. Periodontitis was highly prevalent in all three groups (Table 6).

Table 6. Baseline and follow-up (24 months) oro-dental status findings [mean (SD), no (%)].

	HT group		Alendronate group		Alendronate + HT group	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
N	20	15	18	11	22	14
Number of teeth,	19 ± 8	17 ± 9	16 ± 9	17 ± 10	17 ± 10	17 ± 10
Edentulous	1 (5 %)	1 (7 %)	3 (17 %)	1 (9 %)	4 (18 %)	3 (21 %)
Prosthesis in upper jaw	9 (45 %)	7 (47 %)	11 (61 %)	4 (36 %)	9 (41 %)	6 (43 %)
Prosthesis in lower jaw,	7 (35 %)	6 (40 %)	7 (39%)	4 (36 %)	7 (32 %)	6 (43 %)
DMF index	23 ± 3	24 ± 4 *↑	23 ± 3	22 ± 3	23 ± 4	23 ± 4
DT	0.8 ± 1.3	0.7 ± 1.0	0.4 ± 0.9	0.3 ± 0.5	0.5 ± 0.9	0.7 ± 1.3
FT	13 ± 6	13 ± 7	11 ± 7	12 ± 7	12 ± 8	11 ± 9
Nunber of periodontitis	1 (5 %)	2 (14 %)	1 (7 %)	1 (11 %)	1 (6 %)	-
Mild periodontitis	9 (47 %)	3 (21 %)	4 (27 %)	1 (11 %)	3 (17 %)	-
Severe periodontitis	9 (47 %)	9 (64 %)	10 (67 %)	7 (78 %)	14 (78 %)	11 (100 %)
Nunber of teeth with gingival pockets >6mm,	1.5 ± 2.0	2.1 ± 2.2 *↑	1.6 ± 1.6	2.8 ± 2.0	1.5 ± 1.9	2.8 ± 1.9 *↑
Lesions of mouth mucosa	15 (75%)	12 (80%)	12 (67%)	6 (54%)	13 (59%)	9 (64%)
Signs of TMJ dysfunction	15 (75 %)	10 (67 %)	5 (28 %)	3 (27 %)	10 (45 %)	5 (36 %)

* $p<0.05$

DMF=Diseased, missing, filled teeth

DT=Diseased teeth

FT=Filled teeth

TMJ= Temporomandibular joint

3.2. Salivary findings and matrix metalloproteinase-8

In comparison with baseline values, the resting salivary flow rate decreased by 19.4 % in the alendronate group ($p < 0.05$). The number of women reporting subjective feelings of dry or burning mouth remained the same in each group, with no difference between the groups. The levels of GCF MMP-8 increased in one of the periodontal pockets sampled (A2) in the alendronate group ($p < 0.05$), but in the other pocket (A1) the increase was not significant. In the HRT and combination groups no changes were detected in the concentrations of GCF MMP-8. Salivary protein concentrations remained unchanged in all study groups. No statistically significant changes or inter-group differences were observed in salivary yeast counts (Table 7).

Table 7. Baseline and follow-up (24 months) salivary flow rates, buffering capacity, yeast counts and biochemical constituents [mean+SD or n(%)].

	HT group		Alendronate group		Alendronate +HT group	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
N	20	15	18	11	22	14
Resting salivary flow rate (ml/min)	0.54 ± 0.32	0.65 ± 0.43	0.72 ± 0.46	0.58 ± 0.31*	0.59 ± 0.36	0.70 ± 0.42
Stimulated salivary flow rate (ml/min)	1.54 ± 0.82	1.96 ± 1.06	1.83 ± 0.90	1.71 ± 0.83	1.78 ± 0.71	2.23 ± 1.12
High buffering capacity	14 (78 %)	8 (57 %)	16 (89 %)	8 (89 %)	19 (91 %)	10 (77 %)
Medium buffering capacity	3 (17 %)	5 (36 %)	2 (11 %)	1 (11 %)	2 (10 %)	3 (23 %)
Low buffering capacity	1 (6 %)	1 (7 %)	-	-	-	-
Positive yeast count	14 (78 %)	10 (67 %)	11 (61 %)	4 (36 %)	16 (76 %)	11 (85 %)
MMP-8 A1 (µg/ml)	299 ± 245	296 ± 286	169 ± 115	199 ± 101	212 ± 155	189 ± 189
MMP-8 A2 (µg/ml)	262 ± 205	426 ± 525	208 ± 157	300 ± 185*	136 ± 128	242±213
Albumin (µg/ml)	277 ± 140	262 ± 134	250 ± 143	248 ± 201	252 ± 107	247 ± 144
Salivary total protein (mg/ml)	1.58 ± 0.33	1.55 ± 0.51	1.64 ± 0.35	1.56 ± 0.44	1.55 ± 0.36	1.40 ± 0.43
IgA (µg/ml)	34.1 ± 15.4	28.1 ± 15.1	27.3 ± 7.0	26.5 ± 10.8	28.0 ± 15.2	27.9 ± 33.9
IgG (µg/ml)	26.4 ± 19.3	22.2 ± 18.6	17.3 ± 16.0	13.8 ± 14.1	24.0 ± 14.1	21.7 ± 18.6
IgM (µg/ml)	1.99 ± 1.93	1.87 ± 2.65	1.26 ± 1.00	1.18 ± 1.44	1.10 ± 0.60	0.99 ± 1.74

*p<0.05 for within-group change from baseline

4. Effect of hormone therapy use on the health-related quality of life in the population-based cohort of postmenopausal women (Study IV)

The users of HT were younger (p<0.0001) and healthier in terms of the amount of concomitant medication (p= 0.009) and chronic diseases (p=0.068) and they reported fewer hot flushes (p<0.001). The HT users had higher basic and professional education than the non-users. On average, HT users were taking fewer types of medication than the non-users (2.1 vs. 2.4, p=0.004). The use of medication for depression did not differ between HT users and HT non-users (Table 4).

Of the explanatory variables, the number of types of medication and the number of concomitant chronic diseases, but not age, estrogen use or high education, were statistically significant in the Tobit model (Table 8). The model suggests that the number of types of medication and chronic illnesses have a statistically significant negative effect, but that high education has a positive, but non-significant effect on HRQoL. After standardizing for age, education, number of types of medication, and

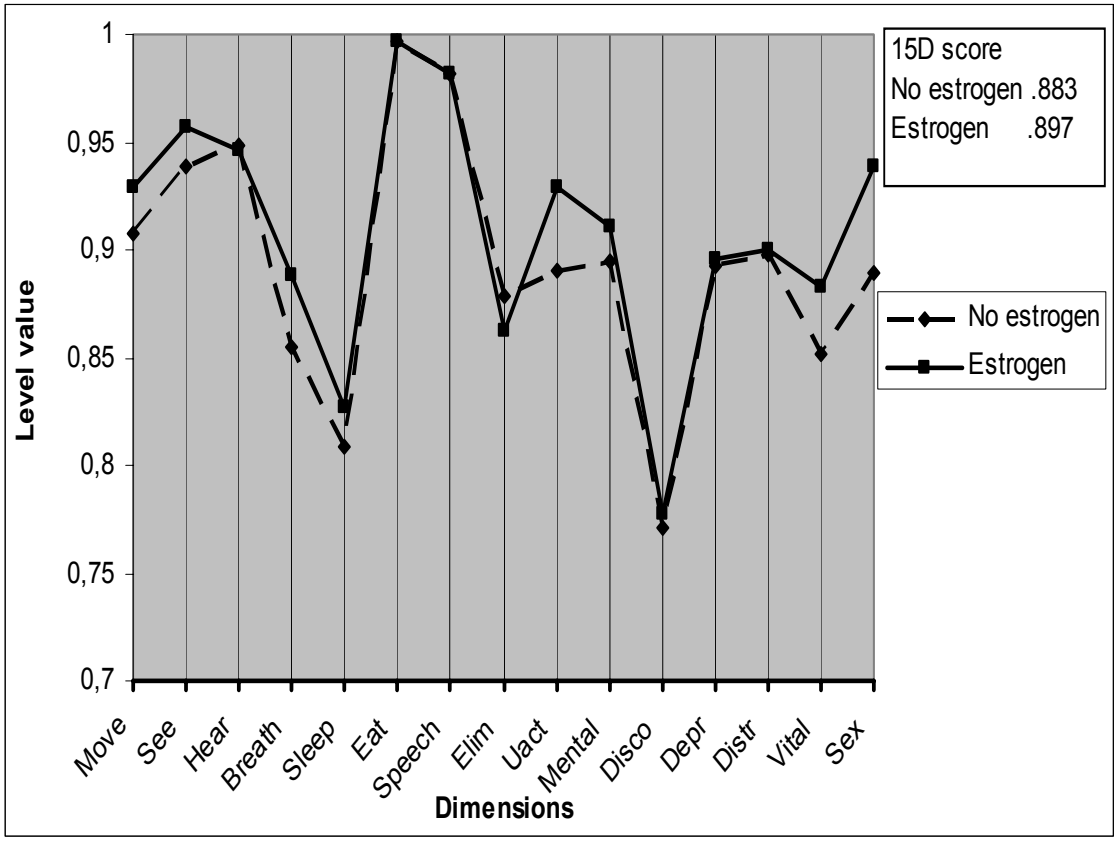
illnesses, the marginal effect of HT use on overall HRQoL on a 0-1 scale was positive (=+0.0053), but statistically non-significant (p=0.21) and less than 0.03, and consequently, not clinically important (Table 8). Indeed, none of the marginal effects of the studied variables exceeded the limit of clinical importance. Without standardization the mean 15D scores of the users and non-users were 0.897 and 0.883, respectively.

Figure 4 shows the 15D scores and profiles of the groups after standardizing for age, education, number of types of medication, and diseases. The hormone users were statistically significantly better off than non-users in the dimensions of usual activities (p=0.0058), vitality (p=0.0076) and sexual activity (p=0.0001), but there were no significant differences in other dimensions, including depression.

Table 8. Results of the Tobit model explaining the variance in the 15D score

Variable	Coefficient	Marginal effect	t-value	p-value
Constant	1.0044	0.9093	21.671	<0.0001
Estrogen use (1=yes, 0=no)	0.0058	0.0053	1.241	0.21
Number of medications	-0.0140	-0.0127	-9.906	<0.0001
Age (years)	-0.0009	-0.0009	-1.404	0.16
Number of chronic diseases	-0.0144	-0.0130	-5.397	<0.0001
High education (1=yes, 0=no)	0.0068	0.0062	1.302	0.19
Sigma (disturbance standard deviation)	0.0811			

Figure 4. The 15D scores and profiles of the HT users and non-users standardized for age, education, number of medications and chronic diseases. Differences favouring HT use were significant for usual activities (p=0.0058), vitality (p=0.0076), and sexual activity (p=0.0001) 15 dimensions:
mobility, vision, hearing, breathing, sleeping, eating, speech, elimination, usual activities, mental function, discomfort and symptoms, depression, distress, vitality and sexual activity.



Discussion

1. Rationale for the study

At the start of the present study estrogens played a major role in the treatment of osteoporosis. Their efficacy in the prevention of osteoporosis (The Writing Group for the PEPI trial 1996) and in the treatment of established osteoporosis (Lufkin *et al* 1992) had been clearly demonstrated. Observational studies indicated that estrogens also prevent CVDs (Grodstein et al 1996) and deaths (Sourander *et al* 1998) and it was believed that they could improve the quality of life of postmenopausal women (Wiklund *et al* 1992, Daly *et al* 1993, Zethraeus *et al* 1997). Elderly women were also generally treated with estrogens, a fact that was still seen in the 35 % share of estrogen users in our population-based cohort of elderly women in the year 2002. However, data on the efficacy and safety of ERT came mainly from younger postmenopausal women. Consequently, we focused the present study on elderly women.

During our investigation the randomized trials HERS and WHI demonstrated that the use of HT in healthy, asymptomatic postmenopausal women lacks overall health benefits and confers several health risks, with breast cancer,VTE and stroke being the most important ones (Hlatky *et al* 2002, Hays *et al* 2003). The increased risk of breast cancer was of the same magnitude as in earlier epidemiological studies (Anonymous 1997). It was also known that current HT use is associated with an increased risk of VTE (Daly *et al* 1996). However, despite the fact that these new results from RCTs greatly reduced the use of HT, postmenopausal women may consider well-being to be a more important factor than the reported health risks and want to start HT. On the other hand, regarding the CVD risk, timing of the start of HT may be very important. The latest evidence from the WHI study indicates that the risk of cardiovascular events is not increased if HT is started between 50 to 59 years of age (HR 0.63, 95% CI 0.36-1.08) (Hsia *et al* 2006). Furthermore, we do not have any data from randomized controlled trials to tell us what kind of long-term CVD risk HT use confers if started at the menopause and continued until the age of 70 to 80 years, for example.

Interestingly, recent observations suggest that postmenopausal women with osteoporosis are at an increased risk of CVD (both coronary events and stroke) that is proportional to the severity of osteoporosis at the time of diagnosis. Treatment of postmenopausal osteoporosis should therefore include consideration of measures to prevent undesirable cardiovascular outcomes (Tanko *et al* 2005), or, at least, not to affect health status negatively. Consequently, studies in which different treatments of osteoporosis (e.g. HT and bisphosphonates) are compared as regards their effects on surrogate markers of CVDs are important. The underlying mechanism by which these two major epidemiologic diseases are linked is not fully understood, but suggested linking factors include regulators of bone turnover, e.g. a matrix GLA protein,

osteocalcin, and osteoprotegerin, proinflammatory cytokines such as IL-6 and TNF-α, oxidized lipids, and NO synthase (Tanko *et al* 2005).

Examination and treatment of osteoporosis is most profitable when directed to women aged 65–70 years (Black 1995). Women with severe osteoporosis or those who have failed to respond optimally to estrogen or bisphosphonate alone might obtain additive benefit when they combine these two antiresorptive agents, with different mechanisms of action. Estrogen inhibits bone resorption by decreasing the activity of osteoclasts and by increasing their apoptosis (Manolagas 2000). Estrogen affects through receptors in the osteoblasts by down-regulating the expression of bone resorbing cytokines, such as interleukins and tumor necrosis factor-α. Bisphosphonates inhibit osteoclasts, and prevent the apoptosis of osteocytes, but the mechanisms by which bisphosphonates inhibit osteoclasts are not totally clear.

We compared the continuous combination of 2 mg estradiol plus 1 mg NETA daily with 10 mg of alendronate daily, separately and in combination for two years. The inclusion of a placebo group was considered unethical, since low BMD combined with advanced age is a straightforward indication for treatment of osteoporosis. A lower dose of continuous combined regimen with 1 mg of estradiol, which is now preferable in this age group, was not commercially available in Finland at the time of the start of the study

2. Effects of hormone therapy , alendronate, and their combination on bone

In the WHI study estrogens reduced the incidence of vertebral and non-vertebral, and also hip fractures in a population that was not selected for BMD or fracture history (Rossouw *et al* 2002, Cauley *et al* 2003). In a randomized placebo-controlled trial estrogen decreased the risk of vertebral fractures in postmenopausal women with established osteoporosis statistically significantly (Lufkin *et al* 1992). In a cohort study HT effectively prevented hip fractures among women older than 75 years (Cauley *et al*. 1995). Estrogens combined with progestins may increase vertebral BMD more than estrogens alone (Christiansen *et al* 1990, Speroff *et al* 1996, Wring group for the PEPI trial 1996, Torgerson *et al* 2001, Nelson *et al* 2002).

Alendronate is the most widely studied bisphosphonate, and it has approximately halved the risk of vertebral and forearm fractures, and also that of hip fractures (Lieberman *et al* 1995, Black *et al* 1996, Karpf *et al* 1997, Black *et al* 2000) in postmenopausal women with osteoporosis, of 55-81 years of age. Besides estrogens and alendronate, only risedronate (McClung *et al* 2001), and in a subgroup analysis also strontium ranelate (Reginster *et al* 2005) have significantly reduced the risk of hip fractures.

In our study of elderly osteoporotic women, two years' combination treatment did not offer an extra benefit over either treatment alone in terms of changes in BMD. DXA was chosen as a tool to measure BMD repeatedly, because it provides good precision (error 1-2%) at a low radiation burden, stable calibration and a short

scanning time (2-5min). Lumbar spine BMD increased similarly in all treatment groups, ranging from 6.8 % to 8.4 % at 12 months and from 9.1 % to 11.2% at 24 months. HT increased femoral neck BMD significantly by 4.9 % and 5.8% at 12 and 24 months respectively. At the latter time point the increase in femoral neck BMD was significantly greater in the HT group than in the other groups. However, the increases in BMD explain only part of the reduction in vertebral fracture risk in response to antiresorptive treatments (4% for raloxifene, 16 % for alendronate, 28 % for risedronate)(Cummings *et al* 2002, Sarkar *et al 2*002, Delmas and Seeman 2004, Sarkar *et al* 2004) and none of the reduction in non-vertebral fracture risk (Delmas and Seeman 2004).

Markers of bone metabolism are sensitive tools when following the effect of antiresorptive treatment of osteoporosis in clinical practice. Their levels change rapidly after the start of treatment and the efficacy of therapy can be evaluated by bone markers in 1 to 3 months, versus1 to 2 years as regards BMD (Garnero and Delmas 2004). Moreover, in comparison with increases of BMD, their decreased levels can explain a greater part of the reduction in vertebral fracture risk in response to antiresorptive treatment. In randomized clinical trials this explanatory share has varied from from 69 to 75 % for raloxifene and from 50 % to 75 % for risedronate (Bjarnasson *et al* 2001, Cummings *et al* 2002, Eastell *et al* 2003). In the present study we measured the urinary N-telopeptide (NTX) of type I collagen as related to creatinine as a marker of cathepsin-K-mediated bone resorption, and the serum aminoterminal propeptide of human type I procollagen (PINP) as a marker of bone formation. The concentrations of both urinary NTX and serum PINP decreased significantly in all three groups. In the HT group the reductions were 50 % to 60 %, being significantly less than those of 70 to 80 % in the other groups. However, it is not known to what extent bone turnover rate can be safely suppressed by antiresorptive agents. Severe suppression of bone turnover may develop during long-term alendronate therapy, resulting in increased susceptibility to, and delayed healing of pathological fractures (Odvina *et al* 2005). Although the co-administration of estrogens or corticosteroids might have been a predisposing factor, this apparent complication also occurred on monotherapy with alendronate (Odvina *et al* 2005). On the other hand, in risedronate-treated patients the reduction of vertebral fracture risk did not increase after reaching a particular threshold in the suppression of bone markers (Eastell 2003). This was not, however, the case for alendronate-treated patients, in which the greater the risk reduction, the lower the marker levels (Bauer *et al* 2004, Bauer *et al* 2006).

In terms of BMD changes, our results deviate from those of previous studies, which mostly have favored the combination of HT and bisphosphonate (alendronate, etidronate, or risedronate) over at least HT alone (Wimalawansa 1995, Harris *et al* 2001). This deviation is not explained by estrogen dose, since equivalent treatments (2 mg estradiol or 0.625 mg CEE) were used in previous studies (Bone *et al* 2000, Tiras

et al 2000, Harris *et al* 2001, Palomba *et al* 2002). As a progestin component of HT we used NETA, which is known to increase bone mass (Christiansen *et al* 1990), but the same regimen was used by Tiras *et al* (2000). In three studies (Lindsay *et al* 1999, Harris *et al* 2001, Greenspan *et al* 2003) medroxyprogesterone acetate was used and in two others (Bone *et al* 2000, Palomba *et al* 2002) no progestin at all was employed. Most importantly, however, our study was aimed at investigation of the effects of treatment of osteoporosis in elderly women with a mean age of 71 years, which is 10 to 20 years higher than in earlier studies (Wimalawansa 1995, Lindsay *et al* 1999, Bone *et al* 2000, Tiras *et al* 2000*,* Harris *et al* 2001, Palomba *et al* 2002) except for that carried out by Greenspan *et al* (2003). It can be reasoned that our elderly subjects represented a group with lower bone turnover rate, which was sufficiently suppressed with one drug alone. Consequently, the combination did not offer any extra benefit. It is well known that after the first few postmenopausal years bone turnover rate goes down before increasing again in late senescence (Mazess 1982, Garnero *et al* 1994). It has been demonstrated that the lower the endogenous estradiol level, the higher the response of BMD to HT in elderly women (Rapuri *et al* 2004). Since serum estradiol concentration is inversely related to age (Rogers *et al* 2002) it can be anticipated that estrogen levels are lower in late than in early postmenopausal women. It is not clear why our results deviated from those of Greenspan *et al* (2003), who also studied elderly women with at mean age of 71 years. In a 3-year study they found (with equivalent estrogen doses) the combination therapy to be superior to HT or alendronate alone, and alendronate to be superior to HT in terms of BMD changes (Greenspan *et al* 2003). The number of women in the study was clearly greater than in ours, but women with osteopenia only were also included and the duration of the study was one year longer than that of the present study.

Collectively, our study shows HT to be as effective as alendronate alone or in combination with HT in increasing BMD of elderly women with osteoporosis. The suppression of bone turnover is somewhat less with HT only, but regarding the long-term safety of antiresorptive treatment this may be a beneficial characteristic of the treatment. Using a drug combination in the treatment of osteoporosis also has an economic aspect, which will be finally resolved only in studies with fracture as an endpoint. However, treatment with alendronate has a fairly good cost-effectiveness ratio (Mobley *et al* 2006), which is strongly related to the age of the patient, with the cost per quality-adjusted life-years (QALYs) decreasing with advanced age (Stevenson *et al* 2005).

3. Effects of hormone therapy, alendronate, and their combination on surrogate markers of cardiovascular diseases

After the onset of menopause the occurrence of CVD rises and by the age of 70 years it is equal in women and men (Carr 2003). Women with osteoporosis are at an increased risk of cardiovascular events (Tanko *et al* 2005). Estrogen has several

favorable effects, that may be protective against cardiovascular diseases, the changes in serum lipids and lipoproteins being most widely studied (The Writing Group for the PEPI trial 1995, Godsland 2001, Davison and Davis 2003). The previous findings have suggested a protective effect of long-term HT on age-related thickening of the intima-media of the carotid artery (Tremollieres *et al* 2000, Hodis *et al* 2001), but in the elderly women with established coronary-artery atherosclerosis HT or ERT have had no significant effect on the progression of atherosclerosis (Hodis *et al* 2003). In recent studies no cardiovascular benefits or even adverse effects have been reported for HT (Grady *et al* 2002, Rossouw *et al* 2002). In the estrogen group of the WHI study the HR associated with CHD was non-significantly decreased (HR 0.91, 95 % CI 0.75-1.12), but was increased by in the estrogen-progestin group (HR 1.29, 95 % CI 1.02-1.63) in comparison to placebo; total CVD risk was also increased in the estrogen-only group by (HR 1.12, 95 % CI 1.01-1.24) (The Women's Health Initiative Steering Committee 2004).

At the vascular level, timing of the start of HT may be critical, depending on the stage of the atherosclerotic process. It can be speculated that before and at early stages of the atherosclerotic process HT might even be protective against disease progression, but at later stages of the process HT becomes harmful. The latest evidence from the WHI study showed, that for women on CEE started at the age of 50 to 59 years, the HR for coronary events was 0.63 (95 % CI 0.36-1.08) compared with 0.95 (95 % CI 0.79-1.16) for all women assigned to receive CEE (Hsia *et al* 2006). For some cognitive domains also, early initation of HT around menopause may be beneficial, while a start in late menopause may be detrimental (MacLennan *et al* 2006).

Bisphosphonates (etidronate, pamidronate, clodronate) have been shown to inhibit the development of experimental atherosclerosis without altering the serum lipid profile, and to inhibit arterial calcification, lipid accumulation and fibrosis (Ylitalo 2000). These three bisphosphonates accumulate extensively in arterial walls and suppress macrophages in atheromatous lesions. In macrophage cultures, bisphosphonates inhibit the cellular accumulation and degradation of atherogenic LDL cholesterol and the formation of foam cells (Ylitalo 2000). Furthermore, etidronate has been shown to decrease the thickening of the carotid arterial wall (Ylitalo 2000).

The most powerful inflammatory surrogate marker of future cardiovascular risk is CRP, and it seems to have a direct proinflammatory effect (Ridker *et al* 1998, Lagrand *et al* 1999, Ridker *et al* 2000, Blake and Ridker 2001, Danesh *et al* 2004). Oral HT has been associated with increased levels of CRP (Ridker *et al* 1999, van Baal *et al* 1999, Luyer *et al* 2001, Prelevic *et al* 2002), whereas transdermal HT has had no effect (Vehkavaara *et al* 2001) or somewhat decreasing effect on serum CRP levels (Sattar et al1999). In our study elevation of the levels of CRP during oral HT was of the same magnitude as in previous studies (Ridker *et al* 1999, van Baal *et al*

1999, Luyer *et al* 2001, Prelevic *et al* 2002). It is, however, an open question whether the rise of CRP during HT use is a real sign of an inflammatory process in the arterial wall, or only a nonspecific marker of liver stimulation (Lakoski *et al* 2005). In our study the latter option was supported by simultaneous rises in the levels of serum CRP and SHBG, a rough marker of liver stimulation, after 6 months of HT, even though no significant correlation between the changes in CRP and SHBG emerged at the individual level. ERT with NETA had only a minor effect on the synthesis of SHBG as was seen in the previous studies (Nugent *et al* 2003). Alendronate did not affect serum CRP or SHBG levels.

Inflammation has a role in the detachment of atherosclerotic plaques that leads to the final occlusion of an artery. Circulating macrophages and lymphocytes become trapped on the vascular wall by adhesion molecules, e.g. E-selectin (Gearing and Newman 1993), a process which may play a role in the development of atherosclerosis (Farzati *et al* 2002). Various forms of HT are known to reduce levels of endothelial adhesion molecules in the peripheral blood (Cushman *et al* 1999, Farzati *et al* 2002), and this effect of HT has been considered protective against CVD (Mendelsohn and Karas1999). In our study oral HT reduced the circulating levels of E-selectin but alendronate did not have any effect on this marker.

4. Effects of hormone therapy, alendronate, and their combination on oral health

Early detection of tissue destruction is desirable to prevent further irreversible loss of connective tissue attachment of teeth and adjacent alveolar bone (Wactawski-Wende *et al* 1996, Tezal *et al* 2000, Wactawski-Wende 2001). Elderly women with osteoporosis are at an increased risk of attachment loss of teeth. The risk for HT users has been reported to be lower and this has been associated with higher BMD (Paganini-Hill 1995, Grodstein *et al* 1998, Krall et *al* 1998, Payne *et al* 1999, Reinhardt *et al*1999, Ronderos *et al* 2000, Hildebolt *et al* 2004). Alveolar crest height and alveolar bone density have been found to be higher in postmenopausal women receiving HT vs. placebo (Civitelly *et al* 2002), and severe clinical attachment loss (11.9 % vs.18.6 %) and alveolar bone loss (20.3 % vs. 34 %) have been reported to be decreased in HT users vs. non-users (Grossi 1998). Calcium and vitamin D supplementation also decrease alveolar bone loss (Krall *et al* 2001, Hildebolt *et al* 2004).

Alendronate have been shown to improve periodontal disease and to suppress bone turnover in postmenopausal women (Jeffcoat 1998, El-Shinnawi and El-Tantawy 2003, Rocha *et al* 2004). Bisphosphonate treatment have been shown to improve the clinical outcome of non-surgical periodontal therapy of chronic periodontitis (Lane *et al* 2005) and it tends to promote bone formation around endosseous implants (Tenenbaum et al 2002). Bisphosphonates (clodronate, alendronate, pamidronate, zoledronate) have been shown to inhibit the catalytic activities of MMPs, including MMP-8, *in vitro* (Teronen *et al* 1999, Heikkilä *et al*

2002), but in the present study alendronate did not down-regulate GCF MMP-8 levels or expression *in vivo*. No effect or a slight tendency to increase GCF MMP-8 levels was observed. Depending on either the destructive (Sorsa *et al* 2004) or anti-inflammatory role (Owen *et al* 2004) of MMP-8 in oral inflammation, this may even be useful. Estrogen has also been shown to have a dose-dependent effect on MMPs and it decreased MMP-1 synthesis in cultures of human osteoblastic cells (Liao and Luo 2001).

In previous studies climacteric symptoms in themselves have been reported as risk factors of burning mouth syndrome (Tarkkila *et al* 2001) independently of HT use and non-use. Hormone therapy has been reported to ameliorate dry mouth feelings, which are very common in elderly women (Hakeberg *et al* 1997, Leimola-Virtanen *et al* 1997, Wardrop *et al* 1989, Friedlander 2002, Eliasson *et al* 2003). Oral dryness is the most significant oral discomfort in women in the menopausal period. The salivary flow rate has been shown to decrease in the menopausal period (women at the age of 50.7 years compared with non-menopausal women at the age of 42.4 years**)** but to increase after the start of HT with alendronate and calcium supplementation in younger postmenopausal women (Yalcin *et al* 2005). The authors concluded that this was the effect of HT, but they did not study the effects of alendronate separately. In our study the significant decrease in the mean resting salivary flow rate in the alendronate group was not desirable from a clinical point of view, because saliva is important to the health of the teeth and oral mucosa. Saliva is the moisturizing and lubricating component of the oral defense system and a decrease in its secretion is often reflected in symptoms of dry and burning mouth, and oral yeast infections (Pajukoski *et al* 2001). In the present study, however, no difference was observed between the study groups in the numbers of subjects with mucosal pathology or positive yeast counts, or in the numbers of women reporting or not reporting disturbing mouth symptoms. In the HT group a trend was seen towards a less frequent positive yeast count but this difference was not statistically significant. The discrepancy between the present and previous studies might in part be due to the higher age of the present subjects.

Salivary protein analyses can be used to measure serum-derived proteins in saliva as markers of the integrity of mouth mucosa, with very small amounts of these proteins being detected in the saliva in healthy subjects (Laine *et al* 1992, Pajukoski *et al* 1997, Meurman *et al* 1998). Treatment with HT and alendronate, alone or in combination, had no effect on oral mucosa in this regard.

Collectively, the only significant finding in this part of our study was the reduced resting saliva flow rate in the alendronate-treated women. Limitations were the small number of participants and the relatively high drop-out rate. Therefore, the results must be interpreted very cautiously.

5. Effect of hormone therapy on the health-related quality of life

We used the 15D questionnaire to measure HRQoL. It is a generic, comprehensive, 15-dimensional, standardized, self-administered measure of HRQoL that can be used both as a profile and a single index score measure. In an earlier study of women younger than those in the present trial (mean age of 62 years) the 15D questionnaire worked as well as the Nottingham Health Profile (NHP) in showing a positive impact of HT (continuously combined estradiol valerate [E2V] plus medroxyprogesterone acetate [MPA] for 6 years) on HRQoL in comparison with age-matched non-users. All six dimensions of the NHP and 13 of the 15 dimensions of the 15D questionnaire were improved, with a clinically significant (>0.03) difference of 0.044 in the general 15D score (Sintonen *et al* 2003). Other questionnaires such as RAND-36, SF-36, SIP and EQ-5D have also been used to measure quality of life related to HT.

The positive effects of HT on climacteric symptoms are well documented. The impact of HT on generic HRQoL has not been so widely studied, but the results have been promising in early menopause (Wiklund *et al* 1992, Zethraeus *et al* 1997, Genazzani *et al* 2002). In women with a mean age of 53 years, four months' treatment with transdermal estrogen combined with MPA for 14 days per month brought an improvement in all dimensions of the NHP, especially the dimensions of sleep and energy (Wiklund *et al* 1992). Low-dose continuously combined HT also improved HRQoL (measured by means of the 15D questionnaire) of younger postmenopausal women (mean 56 years) for whom the relief of menopausal symptoms was the objective of treatment (Ylikangas *et al* 2005). In a cross-sectional study HT users scored better than non-users in most dimensions of HRQoL measured by means of SF-36 and EQ-5D questionnaires, but women of 55 years or older (41 % of HT users and 38 % of non-users) reported lower scores relative to the physical domains, but higher scores in the mental domains as opposed to the younger women. It was also found that QoL was mainly influenced by socio-economic and cultural factors (Genazzani *et al* 2002).

The women in the above mentioned studies were significantly younger than in our study. Women are expected to spend around one-third of their lives in the postmenopausal state, and up to 25 % of elderly women who have been postmenopausal for more than 10 years still suffer from moderate or severe climacteric symptoms (Oldenhave *et al* 1993, Zichella 1993). In our study 4 % of HT users and 7 % of non-users still had hot flushes at least some of the time, and there was a statistically significant difference in the occurrence of these symptoms between the groups. However, we did not include hot flushes as an explanatory variable in our model, since it is a somewhat dependent variable, the effect of which should be incorporated in the 15D score.

Postmenopausal women, making a decision on whether or not to use HT, may consider well-being to be a more important factor than the reported health risks

(HERS; WHI) (Hlatky *et al* 2002, Hays *et al* 2003). Nevertheless, in recent trials, the impact of HT on quality of life has remained unclear. In the WHI trial women assigned to use HT (mean age 63 years) experienced fewer sleep disturbances, better physical functioning and less bodily pain than women who were assigned to use placebo (RAND-36). The difference was statistically, but not clinically significant at one year of treatment, but it disappeared with continuation of treatment for an additional two years (Hays *et al* 2003). In the WHI estrogen-only group CEE had no clinically meaningful effect on HRQoL; one year of treatment with CEE produced a significant small reduction in sleep disturbances but a significant small negative effect on social functioning (Brunner *et al* 2005).

The HERS trial showed mixed effects. Women without hot flushes at entry showed declines in physical measures and those with hot flushes showed improvement in emotional measures when assigned to HT (mean age of 67 years). HRQoL, measured by means of several questionnaires (RAND, depressive symptoms on the Burnam screening scale), was impaired in older women and in those with chronic diseases (diabetes, hypertension, chest pain, heart failure). The negative effects of these factors on QoL outweighed the positive impact of HT (Hlatky *et al* 2002), as did chronic diseases and concomitant medication in the present study.

In the WHI study relief of climacteric symptoms with HT was not associated with improvement in HRQoL during the three years (Hays *et al* 2003). In our cross-sectional study, HT users reported fewer hot flushes than the non-users, as expected, but their general HRQoL was not clinically better. Our study showed that elderly HT users were better off in the dimensions of usual activities, vitality and sexual activity than non-users, but the clinical relevance of this finding might be poor. For example, in terms of sexual activity the difference found originated from four percent of estrogen users being one out of 5 levels better off than non-users.

A limitation of our study is its cross-sectional nature, implying that the groups of estrogen users and non-users were not assigned randomly. To control for or at least alleviate the possible selection effect, the Tobit regression model was used to standardize the groups in terms of important background and clinical characteristics such as age, education, number of medications and chronic diseases. The results suggest that education has a non-significant positive and the number of medication, and chronic diseases, a statistically significant negative effect on HRQoL, but the effect of age, against expectations, non-significant. Obviously the number of types of medication and diseases, detract from the independent significance of age, since they are age-related variables. This turned indeed to be the case, since in a model where the number of types of medication and the number of diseases were removed, the coefficient of age was highly significant ($p=0.0001$).

In our study HT users were more educated and healthier in terms of the number of concomitant chronic diseases and medication, which might cause a healthy user bias as seen in previous observational studies. The use of antidepressants did not

differ between the groups. Therefore, modeling was used to control for or at least alleviate such a bias. Inferring from the facts of no difference in use of antidepressants, and level value on the 15D dimension of depression estrogen deficiency does not seem to increase the susceptibility to depression in this age group.

Collectively, in this population-based study, HT seemed to improve the HRQoL of elderly postmenopausal women slightly in the dimensions of usual activities, vitality and sexual activity, but the overall improvement in HRQoL was neither statistically significant nor clinically important.

Summary and Conclusions

We randomized 90 elderly osteoporotic women between 65 and 80 years of age to receive HT (a continuous combination of oral estradiol plus NETA) or alendronate or their combination for two years and compared the treatments with regard to their effects on bone mineral density and turnover, two surrogate markers of risk of cardiovascular diseases, CRP and E-selectin, as well as oral health.

All the treatments increased BMD at the lumbar spine and the upper femur to a similar extent, with the combination not offering an extra gain. Bone turnover rate was decreased less by HT than by alendronate alone or the combination, but the optimum for the suppression of bone turnover during antiresorptive therapy is unknown

HT but not alendronate increased serum CRP concentrations but the simultaneous rise in serum SHBG levels suggested the change to be a sign of unspecific stimulation of hepatic protein synthesis by estrogens. HT but not alendronate decreased serum E-selectin levels, which may reflect a direct positive effect of HT on endothelial cells. Alendronate did not block the effect of HT on these surrogate markers in the combination group.

Alendronate caused a decrease in the resting salivary flow rate and tended to increase GCF MMP-8 levels. Otherwise, the treatments had no effect on oral health parameters, but firm conclusions were limited by a low participation rate and a high drop-out rate in this part of the study.

In the population-based cohort of 1663 postmenopausal women aged 65-70 years, 585 women were estrogen users and 1078 were non-users. Measured by means of the 15D questionnaire, HT seemed to improve the HRQoL of elderly postmenopausal women significantly in the dimensions of usual activities, vitality and sexual activity, but the overall improvement in HRQoL was neither statistically significant nor clinically important.

In elderly postmenopausal women HT is an effective alternative to treat osteoporosis, but not to improve quality of life or oral health. It has divergent effects on markers of the risk of cardiovascular diseases. Given all the potential risks of coronary heart disease, stroke, thromboembolic events and cancer associated with HT, bisphosphonates might be the first option to start the treatment of postmenopausal osteoporosis in the old age.

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References

Ainamo, J., Barmes, D., Beagrie, G., Cutress, T., Martin, J. and Sardo-Infirri, J. (1982) Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). *Int. Dent. J.,* **32,** 281-291.

Alakangas, A., Selander, K., Mulari, M., Halleen, J., Lehenkari, P., Monkkonen, J., Salo, J. and Vaananen, K. (2002) Alendronate disturbs vesicular trafficking in osteoclasts. *Calcif. Tissue Int.,* **70,** 40-47.

Alhava, E. (2004) Epidemiology of osteoporosis in Finland. *Duodecim,* **120,** 2149-2150.

Ambrose, J.A. and Martinez, E.E. (2002) A new paradigm for plaque stabilization. *Circulation,* **105,** 2000-2004.

Anderson GL, Judd HL, Kaunitz AM, Barad DH, Beresford SA, Pettinger M, Liu J, McNeeley SG, Lopez AM; Women's Health Initiative Investigators. (2003) Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. *JAMA*, **290**(13),1739-48.

Anonymous (1987) World Health Organization. Oral Health Surveys. Basic methods 3rd. Geneva: WHO.

Anonymous (1993) Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am. J. Med.,* **94,** 646-650.

Anonymous (1996) Effects of hormone therapy on bone mineral density: results from the postmenopausal estrogen/progestin interventions (PEPI) trial. The Writing Group for the PEPI. *JAMA,* **276,** 1389-1396.

Anonymous (1997) Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet,* **350,** 1047-1059.

Avis, N.E. and McKinlay, S.M. (1991) A longitudinal analysis of women's attitudes toward the menopause: results from the Massachusetts Women's Health Study. *Maturitas,* **13,** 65-79.

Bachmann, G.A. (1995) Influence of menopause on sexuality. *Int. J. Fertil. Menopausal Stud.,* **40,** 16-22.

Barrett-Connor, E. and Laakso, M. (1990) Ischemic heart disease risk in postmenopausal women. Effects of estrogen use on glucose and insulin levels. *Arteriosclerosis,* **10,** 531-534.

Barrett-Connor, E. and Bush, T.L. (1991) Estrogen and coronary heart disease in women. *JAMA,* **265,** 1861-1867.

Barrett-Connor, E. and Miller, V. (1993) Estrogens, lipids, and heart disease. *Clin. Geriatr. Med.,* **9,** 57-67.

Barrett-Connor, E. (2003) Clinical review 162: cardiovascular endocrinology 3: an epidemiologist looks at hormones and heart disease in women. *J. Clin. Endocrinol. Metab.,* **88,** 4031-4042.

Bauer, D.C., Black, D.M., Garnero, P., Hochberg, M., Ott, S., Orloff, J., Thompson, D.E., Ewing, S.K., Delmas, P.D., Fracture Intervention Trial Study Group (2004) Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. *J. Bone Miner. Res.,* **19,** 1250-1258.

Bauer, D.C., Garnero, P., Hochberg, M.C., Santora, A., Delmas, P., Ewing, S.K., Black, D.M., for the Fracture Intervention Research Group (2006) Pretreatment levels of bone turnover and the antifracture efficacy of alendronate: the fracture intervention trial. *J. Bone Miner. Res.,* **21,** 292-299.

Behr, W. and Barnert, J. (1986) Quantification of bone alkaline phosphatase in serum by precipitation with wheat-germ lectin: a simplified method and its clinical plausibility. *Clin. Chem.,* **32,** 1960-1966.

Bell, N.H. (2001) Advances in the treatment of osteoporosis. *Curr. Drug Targets Immune Endocr Metabol. Disord.,* **1,** 93-102.

Birkedal-Hansen, H. (1993) Role of matrix metalloproteinases in human periodontal diseases. *J. Periodontol.,* **64,** 474-484.

Bischoff-Ferrari, H.A., Conzelmann, M., Dick, W., Theiler, R. and Stahelin, H.B. (2003) Effect of vitamin D on muscle strength and relevance in regard to osteoporosis prevention. *Z. Rheumatol.,* **62,** 518-521.

Bischoff-Ferrari, H.A., Willett, W.C., Wong, J.B., Giovannucci, E., Dietrich, T. and Dawson-Hughes, B. (2005) Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA,* **293,** 2257-2264.

Bjarnason,N.H., Sarkar,S., Duong,T., Mitlak,B., Delmas,P.D., Christiansen,C. (2001) Six and twelve month changes in bone turnover are related to reduction in vertebral fracture risk during 3 years of raloxifene treatment in postmenopausal osteoporosis.*Osteoporos. Int.,* **12**, 922-930.

Black, D.M. (1995) Why elderly women should be screened and treated to prevent osteoporosis. *Am. J. Med.,* **98,** 67S-75S.

Black, D.M., Cummings, S.R., Karpf, D.B., Cauley, J.A., Thompson, D.E., Nevitt, M.C., Bauer, D.C., Genant, H.K., Haskell, W.L., Marcus, R., Ott,S.M., Torner, J.C., Quandt, S.A., Reiss, T.F. and Ensrud, K.E. (1996) Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. *Lancet,* **348,** 1535-1541.

Black, D.M., Thompson, D.E., Bauer, D.C., Ensrud, K., Musliner, T., Hochberg, M.C., Nevitt, M.C., Suryawanshi, S., Cummings, S.R. and Fracture Intervention, T. (2000) Fracture risk reduction with alendronate in women with osteoporosis: the Fracture Intervention Trial. FIT Research Group. *J. Clin. Endocrinol. Metab.,* **85,** 4118-4124.

Blake, G.J. and Ridker, P.M. (2001) High sensitivity C-reactive protein for predicting cardiovascular disease: an inflammatory hypothesis. *Eur. Heart J.,* **22,** 349-352.

Blake, G.J. and Ridker, P.M. (2001) Novel clinical markers of vascular wall inflammation. *Circ. Res.,* **89,** 763-771.

Blumel, J.E., Castelo-Branco, C., Binfa, L., Gramegna, G., Tacla, X., Aracena, B., Cumsille, M.A. and Sanjuan, A. (2000) Quality of life after the menopause: a population study. *Maturitas,* **34,** 17-23.

Bonds, D. E., Lasser, N., Qi, L., Brzyski, R., Caan, B., Heiss, G., Limacher, C., Liu, J.H., Mason, E., Oberman, A, O´Sullivan, M.J., Phillips, L.S., Prineas, R.J. and Tinker, L. (2006) The effects of conjugated equine oestrogen an diabetes incidence: The Women´s Health Initiative randomised trial. *Diabetologia*, **49**, 459-468.

Bone, H.G., Downs, R.W.,Jr, Tucci, J.R., Harris, S.T., Weinstein, R.S., Licata, A.A., McClung, M.R., Kimmel, D.B., Gertz, B.J., Hale, E. and Polvino, W.J. (1997) Dose-response relationships for alendronate treatment in osteoporotic elderly women. Alendronate Elderly Osteoporosis Study Centers. *J. Clin. Endocrinol. Metab.,* **82,** 265-274.

Bone, H.G., Greenspan, S.L., McKeever, C., Bell, N., Davidson, M., Downs, R.W., Emkey, R., Meunier, P.J., Miller, S.S., Mulloy, A.L., Recker, R.R., Weiss, S.R., Heyden, N.,

Musliner, T., Suryawanshi, S., Yates, A.J. and Lombardi, A. (2000) Alendronate and estrogen effects in postmenopausal women with low bone mineral density. Alendronate/Estrogen Study Group. *J. Clin. Endocrinol. Metab.,* **85,** 720-726.

Bone, H.G., Hosking, D., Devogelaer, J.P., Tucci, J.R., Emkey, R.D., Tonino, R.P., Rodriguez-Portales, J.A., Downs, R.W., Gupta, J., Santora, A.C., Liberman,U.A., Alendronate Phase III Osteoporosis Treatment Study,Group. (2004) Ten years' experience with alendronate for osteoporosis in postmenopausal women. *N. Engl. J. Med.,* **350,** 1189-1199.

Braunstein, J.B., Kershner, D.W., Bray, P., Gerstenblith, G., Schulman, S.P., Post, W.S. and Blumenthal, R.S. (2002) Interaction of hemostatic genetics with hormone therapy: new insights to explain arterial thrombosis in postmenopausal women. *Chest,* **121,** 906-920.

Brazier, J., Usherwood, T., Harper, R. and Thomas, K. (1998) Deriving a preference-based single index from the UK SF-36 Health Survey. *J. Clin. Epidemiol.,* **51,** 1115-1128.

Brett, K.M. and Madans, J.H. (1997) Use of postmenopausal hormone replacement therapy: estimates from a nationally representative cohort study. *Am. J. Epidemiol.,* **145,** 536-545.

Brunner, R.L., Gass, M., Aragaki, A., Hays, J., Granek, I., Woods, N., Mason, E., Brzyski, R., Ockene, J., Assaf, A., LaCroix, A., Matthews, K., Wallace, R.,Women's Health Initiative Investigators. (2005) Effects of conjugated equine estrogen on health-related quality of life in postmenopausal women with hysterectomy: results from the Women's Health Initiative Randomized Clinical Trial. *Arch. Intern. Med.,* **165,** 1976-1986.

Calvo, M.S., Eyre, D.R. and Gundberg, C.M. (1996) Molecular basis and clinical application of biological markers of bone turnover. *Endocr. Rev.,* **17,** 333-368.

Campos, H., McNamara, J.R., Wilson, P.W., Ordovas, J.M. and Schaefer, E.J. (1988) Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. *J. Clin. Endocrinol. Metab.,* **67,** 30-35.

Cardozo, L., Bachmann, G., McClish, D., Fonda, D. and Birgerson, L. (1998) Meta-analysis of estrogen therapy in the management of urogenital atrophy in postmenopausal women: second report of the Hormones and Urogenital Therapy Committee. *Obstet. Gynecol.,* **92,** 722-727.

Carey, V.J., Walters, E.E., Colditz, G.A., Solomon, C.G., Willett, W.C., Rosner, B.A., Speizer, F.E. and Manson, J.E. (1997) Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *Am. J. Epidemiol.,* **145,** 614-619.

Carr, M.C. (2003) The emergence of the metabolic syndrome with menopause. *J. Clin. Endocrinol. Metab.,* **88,** 2404-2411.

Cauley, J.A., Seeley, D.G., Ensrud, K., Ettinger, B., Black, D. and Cummings, S.R. (1995) Estrogen replacement therapy and fractures in older women. Study of Osteoporotic Fractures Research Group. *Ann. Intern. Med.,* **122,** 9-16

Cauley, J.A., Robbins, J., Chen, Z., Cummings, S.R., Jackson, R.D., LaCroix, A.Z., LeBoff, M., Lewis, C.E., McGowan, J., Neuner, J., LeBoff, M., Lewis, C.E., McGowan, J., Neuner, J., Pettinger, M., Stefanick, M.L., Wactawski-Wende, J., Watts, N.B., Women's Health Initiative, Investigators. (2003) Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. *JAMA,* **290,** 1729-1738.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P.D. and Meunier, P.J. (1992) Vitamin D3 and calcium to prevent hip fractures in the elderly women. *N. Engl. J. Med.,* **327,** 1637-1642.

Chesnut, C.H.,3rd, Bell, N.H., Clark, G.S., Drinkwater, B.L., English, S.C., Johnson, C.C.,Jr, Notelovitz, M., Rosen, C., Cain, D.F., Flessland, K.A. and Mallinak, N.J. (1997) Hormone replacement therapy in postmenopausal women: urinary N-telopeptide of type I collagen monitors therapeutic effect and predicts response of bone mineral density. *Am. J. Med.,* **102,** 29-37.

Chiechi, L.M., Granieri, M., Lobascio, A., Ferreri, R. and Loizzi, P. (1997) Sexuality in the climacterium. *Clin. Exp. Obstet. Gynecol.,* **24,** 158-159.

Chlebowski, R.T., Hendrix, S.L., Langer, R.D., Stefanick, M.L., Gass, M., Lane, D., Rodabough, R.J., Gilligan, M.A., Cyr, M.G., Thomson, C.A., Khandekar, J., Petrovitch, H. and McTiernan, A., WHI, Investigators. (2003) Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *JAMA,* **289,** 3243-3253.

Christgau, S., Bitsch-Jensen, O., Hanover Bjarnason, N., Gamwell Henriksen, E., Qvist, P., Alexandersen, P. and Bang Henriksen, D. (2000) Serum CrossLaps for monitoring the response in individuals undergoing antiresorptive therapy. *Bone,* **26,** 505-511.

Christiansen, C. and Riis, B.J. (1990) 17 Beta-estradiol and continuous norethisterone: a unique treatment for established osteoporosis in elderly women. *J. Clin. Endocrinol. Metab.,* **71,** 836-841.

Cicinelli, E., Ignarro, L.J., Lograno, M., Matteo, G., Falco, N. and Schonauer, L.M. (1997) Acute effects of transdermal estradiol administration on plasma levels of nitric oxide in postmenopausal women. *Fertil. Steril.,* **67,** 63-66.

Civitelli, R., Pilgram, T.K., Dotson, M., Muckerman, J., Lewandowski, N., Armamento-Villareal, R., Yokoyama-Crothers, N., Kardaris, E.E., Hauser, J., Cohen, S. and Hildebolt, C.F. (2002) Alveolar and postcranial bone density in postmenopausal women receiving hormone/estrogen replacement therapy: a randomized, double-blind, placebo-controlled trial. *Arch. Intern. Med.,* **162,** 1409-1415.

Conard, J., Gompel, A., Pelissier, C., Mirabel, C. and Basdevant, A. (1997) Fibrinogen and plasminogen modifications during oral estradiol replacement therapy. *Fertil. Steril.,* **68,** 449-453.

Cranney, A., Wells, G., Willan, A., Griffith, L., Zytaruk, N., Robinson, V., Black, D., Adachi, J., Shea, B. and Tugwell, P. and Guyatt,G. (2002) Meta-analyses of therapies for postmenopausal osteoporosis. II. Meta-analysis of alendronate for the treatment of postmenopausal women. *Endocr. Rev.,* **23,** 508-516.

Cummings, S.R. and Nevitt, M.C. (1989) A hypothesis: the causes of hip fractures. *J. Gerontol.,* **44,** M107-11.

Cummings SR., Karpf DB., Harris F., Genant HK., Ensrud K., LaCroix AZ., Black DM. (2002) Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. *Am. J. Med*. **112,**281-289.

Cummings, S.R. and Melton, L.J. (2002) Epidemiology and outcomes of osteoporotic fractures. *Lancet,* **359,** 1761-1767.

Cushman, M., Meilahn, E.N., Psaty, B.M., Kuller, L.H., Dobs, A.S. and Tracy, R.P. (1999) Hormone replacement therapy, inflammation, and hemostasis in elderly women. *Arterioscler. Thromb. Vasc. Biol.,* **19,** 893-899.

Cushman, M., Kuller, L.H., Prentice, R., Rodabough, R.J., Psaty, B.M., Stafford, R.S., Sidney, S., Rosendaal, F.R. and Women's Health Initiative, I. (2004) Estrogen plus progestin and risk of venous thrombosis. *JAMA,* **292,** 1573-1580.

Daly, E., Gray, A., Barlow, D., McPherson, K., Roche, M. and Vessey, M. (1993) Measuring the impact of menopausal symptoms on quality of life. *BMJ,* **307,** 836-840.

Daly, E., Vessey, M.P., Hawkins, M.M., Carson, J.L., Gough, P. and Marsh, S. (1996) Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet,* **348,** 977-980.

Danesh, J., Wheeler, J.G., Hirschfield, G.M., Eda, S., Eiriksdottir, G., Rumley, A., Lowe, G.D., Pepys, M.B. and Gudnason, V. (2004) C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N. Engl. J. Med.,* **350,** 1387-1397.

Davison, S. and Davis, S.R. (2003) New markers for cardiovascular disease risk in women: impact of endogenous estrogen status and exogenous postmenopausal hormone therapy. *J. Clin. Endocrinol. Metab.,* **88,** 2470-2478.

Dawson-Hughes, B., Harris, S.S., Krall, E.A. and Dallal, G.E. (1997) Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N. Engl. J. Med.,* **337,** 670-676.

Delmas, P.D. (1992) Clinical use of biochemical markers of bone remodeling in osteoporosis. *Bone,* **13,** S17-21.

Delmas, P.D. and Seeman, E. (2004) Changes in bone mineral density explain little of the reduction in vertebral or nonvertebral fracture risk with anti-resorptive therapy. *Bone,* **34,** 599-604.

Devogelaer, J.P. (1998) A risk-benefit assessment of alendronate in the treatment of involutional osteoporosis. *Drug Saf.,* **19,** 141-154.

Do, K.A., Green, A., Guthrie, J.R., Dudley, E.C., Burger, H.G. and Dennerstein, L. (2000) Longitudinal study of risk factors for coronary heart disease across the menopausal transition. *Am. J. Epidemiol.,* **151,** 584-593.

Dursun, N., Dursun, E. and Yalcin, S. (2001) Comparison of alendronate, calcitonin and calcium treatments in postmenopausal osteoporosis. *Int. J. Clin. Pract.,* **55,** 505-509.

Eastell, R., Barton, I., Hannon, R.A., Chines, A., Garnero, P. and Delmas, P.D. (2003) Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. *J. Bone Miner. Res.,* **18,** 1051-1056.

Ebeling, P.R., Peterson, J.M. and Riggs, B.L. (1992) Utility of type I procollagen propeptide assays for assessing abnormalities in metabolic bone diseases. *J. Bone Miner. Res.,* **7,** 1243-1250.

Ekstrom, H. and Hovelius, B. (2000) Quality of life and hormone therapy in women before and after menopause. *Scand. J. Prim. Health Care,* **18,** 115-121.

Eliasson, L., Carlen, A., Laine, M. and Birkhed, D. (2003) Minor gland and whole saliva in postmenopausal women using a low potency oestrogen (oestriol). *Arch. Oral Biol.,* **48,** 511-517.

El-Shinnawi, U.M. and El-Tantawy, S.I. (2003) The effect of alendronate sodium on alveolar bone loss in periodontitis (clinical trial). *J. Int. Acad. Periodontol.,* **5,** 5-10.

Epstein, S. (2000) Postmenopausal osteoporosis: fracture consequences and treatment efficacy vary by skeletal site. *Aging Clin. Exp. Res.,* **12,** 330-341.

Erkkola, R. (2004) Hormonihoidon ”tuho ja nousu”. *Suom Lääkäril. ,***59**, 2732-2735.

Espeland, M.A., Hogan, P.E., Fineberg, S.E., Howard, G., Schrott, H., Waclawiw, M.A. and Bush, T.L. (1998) Effect of postmenopausal hormone therapy on glucose and insulin concentrations. PEPI Investigators. Postmenopausal Estrogen/Progestin Interventions. *Diabetes Care,* **21,** 1589-1595.

Ettinger, B., Pressman, A. and Silver, P. (1999) Effect of age on reasons for initiation and discontinuation of hormone replacement therapy. *Menopause,* **6,** 282-289.

Ettinger, M.P. (2003) Aging bone and osteoporosis: strategies for preventing fractures in the elderly. *Arch. Intern. Med.,* **163,** 2237-2246.Eyre, D.R. (1997) Bone biomarkers as tools in osteoporosis management. *Spine,* **22,** 17S-24S.

Farzati, A., Esposito, K., Colacurci, N., Fornaro, F., Chiantera, V. and Farzati, B. (2002) Effects of transdermal hormone replacement therapy on levels of soluble P- and E-selectin in postmenopausal healthy women. *Fertil. Steril.,* **77,** 476-480.

Fisher, J.E., Rodan, G.A. and Reszka, A.A. (2000) In vivo effects of bisphosphonates on the osteoclast mevalonate pathway. *Endocrinology,* **141,** 4793-4796.

Friedlander, A.H. (2002) The physiology, medical management and oral implications of menopause. *J. Am. Dent. Assoc.,* **133,** 73-81.

Gambacciani, M., Monteleone, P., Vitale, C., Silvestri, A., Fini, M., Genazzani, A.R. and Rosano, G.M. (2002) Dydrogesterone does not reverse the effects of estradiol on endothelium-dependant vasodilation in postmenopausal women: a randomised clinical trial. *Maturitas,* **43,** 117-123.

Garnero, P., Shih, W.J., Gineyts, E., Karpf, D.B. and Delmas, P.D. (1994) Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J. Clin. Endocrinol. Metab.,* **79,** 1693-1700.

Garnero, P. and Delmas, P.D. (1998) Biochemical markers of bone turnover. Applications for osteoporosis. *Endocrinol. Metab. Clin. North Am.,* **27,** 303-323.

Garnero, P., Ferreras, M., Karsdal, M.A., Nicamhlaoibh, R., Risteli, J., Borel, O., Qvist, P., Delmas, P.D., Foged, N.T. and Delaisse, J.M. (2003) The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. *J. Bone Miner. Res.,* **18,** 859-867.

Garnero, P. and Delmas, P.D. (2004) Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women. *J. Musculoskelet. Interact.,* **4,** 50-63.

Gearing, A.J. and Newman, W. (1993) Circulating adhesion molecules in disease. *Immunol. Today,* **14,** 506-512.

Genant, H.K., Baylink, D.J. and Gallagher, J.C. (1989) Estrogens in the prevention of osteoporosis in postmenopausal women. *Am. J. Obstet. Gynecol.,* **161,** 1842-1846.

Genazzani, A.R., Nicolucci, A., Campagnoli, C., Crosignani, P., Nappi, C., Serra, G.B., Bottiglioni, E., Cianci, A., De Aloysio, D., Donati Sarti, C., Gambacciani, M., Monteleone, P., Genazzani, A.D., Guaschino., S., Palumbo, G., Petraglia, F., Schonauer, S., Volpe, A., Di Paolantonio, T., Nagni, M., Tempesta, A. and Coronel G.A. (2002) Assessment of the QoL in Italian menopausal women: comparison between HRT users and non-users. *Maturitas,* **42,** 267-280.

Godsland, I.F. (2001) Effects of postmenopausal hormone replacement therapy on lipid, lipoprotein, and apolipoprotein (a) concentrations: analysis of studies published from 1974-2000. *Fertil. Steril.,* **75,** 898-915.

Golub, L.M., Lee, H.M., Greenwald, R.A., Ryan, M.E., Sorsa, T., Salo, T. and Giannobile, W.V. (1997) A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm. Res.,* **46,** 310-319.

Grady, D., Gebretsadik, T., Kerlikowske, K., Ernster, V. and Petitti, D. (1995) Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obstet. Gynecol.,* **85,** 304-313.

Grady, D., Herrington, D., Bittner, V., Blumenthal, R., Davidson, M., Hlatky, M., Hsia, J., Hulley, S., Herd, A., Khan, S., Newby, L.K., Waters, D., Vittinghoff, E. and Wenger, N., for the HERS Research Group. (2002) Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). *JAMA,* **288,** 49-57.

Greenspan, S., Bankhurst, A, Bell, N. (1998) Effects of alendronate and estrogen alone and in combination on bone mass and turnover in postmenopausal osteoporosis. *J Bone Miner Res,* S174.

Greenspan, S.L., Resnick, N.M., Parker, R.A. and ScD (2003) Combination Therapy With Hormone Replacement and Alendronate for Prevention of Bone Loss in Elderly Women: A Randomized Controlled Trial. *JAMA,* **289,** 2525-2533.

Greenwald MW, Gluck OS, Lang E, Rakov V. (2005) Oral hormone therapy with 17beta-estradiol and 17beta-estradiol in combination with norethindrone acetate in the prevention of bone loss in early postmenopausal women: dose-dependent effects. *Menopause*,**12**(6):741-8.

Grodstein, F., Colditz, G.A. and Stampfer, M.J. (1996) Post-menopausal hormone use and tooth loss: a prospective study. *J. Am. Dent. Assoc.,* **127,** 370-377.

Grodstein, F., Stampfer, M.J., Manson, J.E., Colditz, G.A., Willett, W.C., Rosner, B., Speizer, F.E. and Hennekens, C.H. (1996) Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N. Engl. J. Med.,* **335,** 453-461.

Grodstein, F., Colditz, G.A. and Stampfer, M.J. (1998) Tooth loss and hormone use in postmenopausal women. *Compend Contin. Educ. Dent. Suppl.***,** S9-16.

Grossi, S.G. (1998) Effect of estrogen supplementation on periodontal disease. *Compend Contin. Educ. Dent. Suppl.,* S30-6.

Guanabens, N., Pares, A., Navasa, M., Martinez de Osaba, M.J., Hernandez, M.E., Munoz, J. and Rodes, J. (1994) Cyclosporin A increases the biochemical markers of bone remodeling in primary biliary cirrhosis. *J. Hepatol.,* **21,** 24-28.

Hakeberg, M., Berggren, U., Hagglin, C. and Ahlqwist, M. (1997) Reported burning mouth symptoms among middle-aged and elderly women. *Eur. J. Oral Sci.,* **105,** 539-543.

Halleen, J.M., Alatalo, S.L., Janckila, A.J., Woitge, H.W., Seibel, M.J. and Vaananen, H.K. (2001) Serum tartrate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. *Clin. Chem.,* **47,** 597-600.

Hanemaaijer, R., Sorsa, T., Konttinen, Y.T., Ding, Y., Sutinen, M., Visser, H., van Hinsbergh, V.W., Helaakoski, T., Kainulainen, T., Ronka, H., Tschesche, H. and Salo, T. (1997) Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J. Biol. Chem.,* **272,** 31504-31509.

Harris, S.T., Eriksen, E.F., Davidson, M., Ettinger, M.P., Moffett Jr, A.H.,Jr, Baylink, D.J., Crusan, C.E. and Chines, A.A. (2001) Effect of combined risedronate and hormone replacement therapies on bone mineral density in postmenopausal women. *J. Clin. Endocrinol. Metab.,* **86,** 1890-1897.

Hawthorne, G., Richardson, J. and Day, N.A. (2001) A comparison of the Assessment of Quality of Life (AQoL) with four other generic utility instruments. *Ann. Med.,* **33,** 358-370.

Hays, J., Ockene, J.K., Brunner, R.L., Kotchen, J.M., Manson, J.E., Patterson, R.E., Aragaki, A.K., Shumaker, S.A., Brzyski, R.G., LaCroix, A.Z., Granek, I.A. and Valanis, B.G., WHI, Investigators. (2003) Effects of estrogen plus progestin on health-related quality of life. *N. Engl. J. Med.,* **348,** 1839-1854.

Heikkilä, P., Teronen, O., Moilanen, M., Konttinen, Y.T., Hanemaaijer, R., Laitinen, M., Maisi, P., van der Pluijm, G., Bartlett, J.D., Salo, T. and Sorsa, T. (2002) Bisphosphonates inhibit stromelysin-1 (MMP-3), matrix metalloelastase (MMP-12), collagenase-3 (MMP-13) and enamelysin (MMP-20), but not urokinase-type plasminogen activator, and diminish invasion and migration of human malignant and endothelial cell lines. *Anticancer Drugs,* **13,** 245-254.

Heikkinen, J., Vaheri, R., Kainulainen, P. and Timonen, U. (2000) Long-term continuous combined hormone replacement therapy in the prevention of postmenopausal bone loss: a comparison of high- and low-dose estrogen-progestin regimens. *Osteoporos. Int.,* **11,** 929-937.

Hellstein, J.W. and Marek, C.L. (2004) Bis-phossy jaw, phossy jaw, and the 21st century: bisphosphonate-associated complications of the jaws. *J. Oral Maxillofac. Surg.,* **62,** 1563-1565.

Hellstein, J.W. and Marek, C.L. (2005) Bisphosphonate osteochemonecrosis (bis-phossy jaw): is this phossy jaw of the 21st century? *J. Oral Maxillofac. Surg.,* **63,** 682-689.

Hewitt, R.E., Lissina, A., Green, A.E., Slay, E.S., Price, D.A. and Sewell, A.K. (2005) The bisphosphonate acute phase response: rapid and copious production of proinflammatory cytokines by peripheral blood gd T cells in response to aminobisphosphonates is inhibited by statins. *Clin. Exp. Immunol.,* **139,** 101-111.

Hildebolt, C.F., Pilgram, T.K., Dotson, M., Armamento-Villareal, R., Hauser, J., Cohen, S. and Civitelli, R. (2004) Estrogen and/or calcium plus vitamin D increase mandibular bone mass. *J. Periodontol.,* **75,** 811-816.

Hlatky MA. Boothroyd D. Vittinghoff E. Sharp P. Whooley MA. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group (2002) Quality-of-life and depressive symptoms in postmenopausal women after receiving hormone therapy: results from the Heart and Estrogen/Progestin Replacement Study (HERS) trial. *JAMA,* **287,** 591-597.

Hodis, H.N., Wendy, J.M., Lobo, R.A., Shoupe, D., Sevanian, A., Mahrer, P.R., Selzer, R.H., Chao-ran, L., Ci-hua, L. and Azen, S.P., for the Estrogen in the Prevention of Atherosclerosis Trial Research Group (2001) Estrogen in the Prevention of Atheroclerosis. *Ann. Intern. Med*., **135**, 939-953.

Hodis, H.N., Marck, W.J., Azen, S.P., Lobo, R.A., Shoupe, D., Mahrer, P.R., Faxon, D.P., Cashin-Hemphill, L., Sanmarco, M.E., French, W.J., Shook, T.L., Gaarder, T.D., Mehra, A.O., Rabbani, R., Sevanian, A., Shil, A.B., Torres, M., Vogelbach, K.H. and Selzer, R.H.(2003) Hormone Therapy and the Progression of Coronary-Artery Atherosclerosis in Postmenopausal Women. *N. Engl. J. Med.,* **349**,535-545

Hogeveen, K.N., Cousin, P., Pugeat, M., Dewailly, D., Soudan, B. and Hammond, G.L. (2002) Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. *J. Clin. Invest.,* **109,** 973-981.

Hosking, D., Chilvers, C.E., Christiansen, C., Ravn, P., Wasnich, R., Ross, P., McClung, M., Balske, A., Thompson, D., Daley, M., Yates, B.A. and Yates, A. (1998) Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. Early Postmenopausal Intervention Cohort Study Group. *N. Engl. J. Med.,* **338,** 485-492.

Hsia, J., Langer, R.D., Manson, J.E.D.H., Kuller, L.D.H., Johnson, K.C., Hendrix, S.L.D.O., Pettinger, M., Heckbert, S.R., Greep, N., Crawford, S., Eaton, C.B., Kostis, J.B., Caralis, P. and Prentice, R., WHI Investigators (2006) Conjugated Equine Estrogens and Coronary Heart Disease: The Women's Health Initiative. *Arch. Intern. Med.,* **166,** 357-365.

Hu, G., Qiao, Q., Tuomilehto, J., Balkau, B., Borch-Johnsen, K., Pyorala, K. and DECODE Study, G. (2004) Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch. Intern. Med.,* **164,** 1066-1076.

Hui, S.L., Slemenda, C.W. and Johnston, C.C.,Jr (1988) Age and bone mass as predictors of fracture in a prospective study. *J. Clin. Invest.,* **81,** 1804-1809.

Hulley,S., Grady,D., Bush,T., Furberg,C., Herrington,D., Riggs,B., Vittinghoff,E. (1998) Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA*, **280**, 605-613.

Humphries, K.H. and Gill, S. (2003) Risks and benefits of hormone replacement therapy: the evidence speaks. *CMAJ,* **168,** 1001-1010.

Hunt, S.M., McKenna, S.P., McEwen, J., Williams, J. and Papp, E. (1981) The Nottingham Health Profile: subjective health status and medical consultations. *Soc. Sci. Med. [A],* **15,** 221-229.

Isles, C.G., Hole, D.J., Hawthorne, V.M. and Lever, A.F. (1992) Relation between coronary risk and coronary mortality in women of the Renfrew and Paisley survey: comparison with men. *Lancet,* **339,** 702-706.

Jeffcoat, M.K. (1998) Osteoporosis: a possible modifying factor in oral bone loss. *Ann. Periodontol.,* **3,** 312-321.

Jensen, J., Nilas, L. and Christiansen, C. (1990) Influence of menopause on serum lipids and lipoproteins. *Maturitas,* **12,** 321-331.

Jick, H., Derby, L.E., Myers, M.W., Vasilakis, C. and Newton, K.M. (1996) Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet,* **348,** 981-983.

Kahn, S.M., Hryb, D.J., Nakhla, A.M., Romas, N.A. and Rosner, W. (2002) Sex hormone-binding globulin is synthesized in target cells. *J. Endocrinol.,* **175,** 113-120.

Kalme, T., Koistinen, H., Loukovaara, M., Koistinen, R. and Leinonen, P. (2003) Comparative studies on the regulation of insulin-like growth factor-binding protein-1 (IGFBP-1) and sex hormone-binding globulin (SHBG) production by insulin and insulin-like growth factors in human hepatoma cells. *J. Steroid Biochem. Mol. Biol.,* **86,** 197-200.

Karjalainen, A., Paassilta, M., Heikkinen, J., Backstrom, A.C., Savolainen, M. and Kesaniemi, Y.A. (2001) Effects of peroral and transdermal oestrogen replacement therapy on glucose and insulin metabolism. *Clin. Endocrinol.,* **54,** 165-173.

Karpf, D.B., Shapiro, D.R., Seeman, E., Ensrud, K.E., Johnston, C.C.,Jr, Adami, S., Harris, S.T., Santora, A.C.,2nd, Hirsch, L.J., Oppenheimer, L. and Thompson, D. (1997) Prevention of nonvertebral fractures by alendronate. A meta-analysis. Alendronate Osteoporosis Treatment Study Groups. *JAMA,* **277,** 1159-1164.

Kauppinen, R., Sintonen, H., Vilkka, V., Pekurinen, M. and Tukiainen, H. (1998) Quality-of-life measures and clinical parameters in asthmatics during three year follow-up. *Monaldi Arch. Chest Dis.,* **53,** 400-404.

Keating, N.L., Cleary, P.D., Rossi, A.S., Zaslavsky, A.M. and Ayanian, J.Z. (1999) Use of hormone replacement therapy by postmenopausal women in the United States. *Ann. Intern. Med.,* **130,** 545-553.

Kennedy, G., McLaren, M., Belch, J.J. and Seed, M. (1999) Elevated levels of sE-selectin in post-menopausal females are decreased by hormone replacement therapy to levels observed in pre-menopausal females. *Thromb. Haemost.,* **82,** 1433-1436.

Khosla, S., Riggs, B.L., Robb, R.A., Camp, J.J., Achenbach, S.J., Oberg, A.L., Rouleau, P.A. and Melton, L.J.,3rd (2005) Relationship of volumetric bone density and structural parameters at different skeletal sites to sex steroid levels in women. *J. Clin. Endocrinol. Metab.,* **90,** 5096-5103.

Koh, K.K., Mincemoyer, R., Bui, M.N., Csako, G., Pucino, F., Guetta, V., Waclawiw, M. and Cannon, R.O.,3rd (1997) Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. *N. Engl. J. Med.,* **336,** 683-690.

Komulainen, M., Kröger, H., Tuppurainen, M.T., Heikkinen, A.M., Alhava, E., Honkanen, R., Jurvelin, J. and Saarikoski, S. (1999) Prevention of femoral and lumbar bone loss with hormone replacement therapy and vitamin D3 in early postmenopausal women: a population-based 5-year randomized trial. *J. Clin. Endocrinol. Metab.* **84**:546-552.

Krall, E.A., Dawson-Hughes, B., Hannan, M.T. and Kiel, D.P. (1998) Postmenopausal estrogen replacement and tooth retention. *Compend Contin. Educ. Dent. Suppl.***,** S17-22.

Krall, E.A., Wehler, C., Garcia, R.I., Harris, S.S. and Dawson-Hughes, B. (2001) Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am. J. Med.,* **111,** 452-456.

Kroger, H. and Reeve, J. (1998) Diagnosis of osteoporosis in clinical practice. *Ann. Med.,* **30,** 278-287.

Lagrand, W.K., Visser, C.A., Hermens, W.T., Niessen, H.W., Verheugt, F.W., Wolbink, G.J. and Hack, C.E. (1999) C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation,* **100,** 96-102.

Laine, P., Meurman, J.H., Tenovuo, J., Murtomaa, H., Lindqvist, C., Pyrhönen, S. and Teerenhovi, L. (1992) Salivary flow and composition in lymphoma patients before, during and after treatment with cytostatic drugs. *Eur. J. Cancer. B. Oral Oncol.,* **28B,** 125-128.

Laine, M. and Leimola-Virtanen, R. (1996) Effect of hormone replacement therapy on salivary flow rate, buffer effect and pH on perimenopausal and postmenopausal women. *Arch. Oral Biol.,* **41,** 91-96.

Lakoski, S.G., Brosnihan, B. and Herrington, D.M. (2005) Hormone therapy, C-reactive protein, and progression of atherosclerosis: data from the Estrogen Replacement on Progression of Coronary Artery Atherosclerosis (ERA) trial. *Am. Heart J.,* **150,** 907-911.

Lane, N., Armitage, G.C., Loomer, P., Hsieh, S., Majumdar, S., Wang, H.Y., Jeffcoat, M. and Munoz, T. (2005) Bisphosphonate therapy improves the outcome of conventional periodontal treatment: results of a 12-month, randomized, placebo-controlled study. *J. Periodontol.,* **76,** 1113-1122.

Lauhio, A., Salo, T., Ding, Y., Konttinen, Y.T., Nordström, D., Tschesche, H., Lähdevirta, J., Golub, L.M. and Sorsa, T. (1994) In vivo inhibition of human neutrophil collagenase (MMP-8) activity during long-term combination therapy of doxycycline and non-steroidal anti-inflammatory drugs (NSAID) in acute reactive arthritis. *Clin. Exp. Immunol.,* **98,** 21-28.

Leimola-Virtanen, R., Helenius, H. and Laine, M. (1997) Hormone replacement therapy and some salivary antimicrobial factors in post- and perimenopausal women. *Maturitas,* **27,** 145-151.

Leimola-Virtanen, R., Salo, T., Toikkanen, S., Pulkkinen, J. and Syrjänen, S. (2000) Expression of estrogen receptor (ER) in oral mucosa and salivary glands. *Maturitas,* **36,** 131-137.

Liao, E.Y. and Luo, X.H. (2001) Effects of 17beta-estradiol on the expression of matrix metalloproteinase-1, -2 and tissue inhibitor of metalloproteinase-1 in human osteoblast-like cell cultures. *Endocrine,* **15,** 291-295.

Liberman, U.A., Weiss, S.R., Broll, J., Minne, H.W., Quan, H., Bell, N.H., Rodriguez-Portales, J., Downs, R.W.,Jr, Dequeker, J. and Favus, M. (1995) Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. *N. Engl. J. Med.,* **333,** 1437-1443.

Liede, K.E., Haukka, J.K., Hietanen, J.H., Mattila, M.H., Ronka, H. and Sorsa, T. (1999) The association between smoking cessation and periodontal status and salivary proteinase levels. *J. Periodontol.,* **70,** 1361-1368.

Lindsay, R. and Tohme, J.F. (1990) Estrogen treatment of patients with established postmenopausal osteoporosis. *Obstet. Gynecol.,* **76,** 290-295.

Lindsay, R., Cosman, F., Lobo, R.A., Walsh, B.W., Harris, S.T., Reagan, J.E., Liss, C.L., Melton, M.E. and Byrnes, C.A. (1999) Addition of alendronate to ongoing hormone replacement therapy in the treatment of osteoporosis: a randomized, controlled clinical trial. *J. Clin. Endocrinol. Metab.,* **84,** 3076-3081.

Lippi, G., Braga, V., Adami, S. and Guidi, G. (1998) Modification of serum apolipoprotein A-I, apolipoprotein B and lipoprotein(a) levels after bisphosphonates-induced acute phase response. *Clin. Chim. Acta,* **271,** 79-87.

Lips, P. (1997) Epidemiology and predictors of fractures associated with osteoporosis. *Am. J. Med.,* **103,** 3S-8S.

Lobo, R.A. (1991) Clinical review 27: Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. *J. Clin. Endocrinol. Metab.,* **73,** 925-930.

Looker, A.C., Bauer, D.C., Chesnut, C.H.,3rd, Gundberg, C.M., Hochberg, M.C., Klee, G., Kleerekoper, M., Watts, N.B. and Bell, N.H. (2000) Clinical use of biochemical markers of bone remodeling: current status and future directions. *Osteoporos. Int.,* **11,** 467-480.

Lufkin, E.G., Wahner, H.W., O'Fallon, W.M., Hodgson, S.F., Kotowicz, M.A., Lane, A.W., Judd, H.L., Caplan, R.H. and Riggs, B.L. (1992) Treatment of postmenopausal osteoporosis with transdermal estrogen. *Ann. Intern. Med.,* **117,** 1-9.

Luyer, M.D., Khosla, S., Owen, W.G. and Miller, V.M. (2001) Prospective randomized study of effects of unopposed estrogen replacement therapy on markers of coagulation and inflammation in postmenopausal women. *J. Clin. Endocrinol. Metab.,* **86,** 3629-3634.

MacKeigan, L.D. and Pathak, D.S. (1992) Overview of health-related quality-of-life measures. *Am. J. Hosp. Pharm.,* **49,** 2236-2245.

MacLennan, A., Lester, S. and Moore, V. (2001) Oral estrogen replacement therapy versus placebo for hot flushes: a systematic review. *Climacteric,* **4,** 58-74.

MacLennan AH, Henderson VW, Paine BJ, Mathias J, Ramsay EN, Ryan P, Stocks NP, Taylor AW. (2006) Hormone therapy, timing of initiation, and cognition in women aged older than 60 years: the REMEMBER pilot study. Menopause, **13**, 28-36.

Manolagas, S.C. (2000) Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr. Rev.,* **21,** 115-137.

Manson, J.E. and Martin, K.A. (2001) Clinical practice. Postmenopausal hormone-replacement therapy. *N. Engl. J. Med.,* **345,** 34-40.

Mäntylä, P., Stenman, M., Kinane, D.F., Tikanoja, S., Luoto, H., Salo, T. and Sorsa, T. (2003) Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J. Periodontal. Res.,* **38,** 436-439.

Margolis, S. (1990) Clinical review: Diagnosis and management of abnormal plasma lipids. *J. Clin. Endocrinol. Metab.,* **70,** 821-825.

Masaki, T. (1993) Endothelins: homeostatic and compensatory actions in the circulatory and endocrine systems. *Endocr. Rev.,* **14,** 256-268.

Matthews, K.A., Wing, R.R., Kuller, L.H., Meilahn, E.N. and Plantinga, P. (1994) Influence of the perimenopause on cardiovascular risk factors and symptoms of middle-aged healthy women. *Arch. Intern. Med.,* **154,** 2349-2355.

Mazess, R.B. (1982) On aging bone loss. *Clin. Orthop.,* 239-252.

McClung, M.R., Geusens, P., Miller, P.D., Zippel, H., Bensen, W.G., Roux, C., Adami, S., Fogelman, I., Diamond, T. and Eastell, R., et al (2001) Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *N. Engl. J. Med.,* **344,** 333-340.

McNagny, S.E. (1999) Prescribing hormone replacement therapy for menopausal symptoms. *Ann. Intern. Med.,* **131,** 605-616.

Melkko, J., Niemi, S., Risteli, L. and Risteli, J. (1990) Radioimmunoassay of the carboxyterminal propeptide of human type I procollagen. *Clin. Chem.,* **36,** 1328-1332.

Melkko, J., Kauppila, S., Niemi, S., Risteli, L., Haukipuro, K., Jukkola, A. and Risteli, J. (1996) Immunoassay for intact amino-terminal propeptide of human type I procollagen. *Clin. Chem.,* **42,** 947-954.

Melton, L.J.,3rd (1995) How many women have osteoporosis now? *J. Bone Miner. Res.,* **10,** 175-177.

Mendelsohn, M.E. and Karas, R.H. (1999) The protective effects of estrogen on the cardiovascular system. *N. Engl. J. Med.,* **340,** 1801-1811.

Meurman, J.H. and Rantonen, P. (1994) Salivary flow rate, buffering capacity, and yeast counts in 187 consecutive adult patients from Kuopio, Finland. *Scand. J. Dent. Res.,* **102,** 229-234.

Meurman, J.H., Collin, H.L., Niskanen, L., Toyry, J., Alakuijala, P., Keinanen, S. and Uusitupa, M. (1998) Saliva in non-insulin-dependent diabetic patients and control subjects: The role of the autonomic nervous system. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.,* **86,** 69-76.

Mizunuma H, Shiraki M, Shintani M, Gorai I, Makita K, Itoga S, Mochizuki Y, Mogi H, Iwaoki Y, Kosha S, Yasui T, Ishihara O, Kurabayashi T, Kasuga Y, Hayashi K. (2006) Randomized trial comparing low-dose hormone replacement therapy and HRT plus 1alpha-OH-vitamin D3 (alfacalcidol) for treatment of postmenopausal bone loss. *J Bone Miner Metab*., **24**,11-5.

Mobley, L.R., Hoerger, T.J., Wittenborn, J.S., Galuska, D.A. and Rao, J.K. (2006) Cost-effectiveness of osteoporosis screening and treatment with hormone replacement therapy, raloxifene, or alendronate. *Med. Decis. Making,* **26,** 194-206.

Moncada, S., Palmer, R.M. and Higgs, E.A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.,* **43,** 109-142.

Monroe, D.G., Getz, B.J., Johnsen, S.A., Riggs, B.L., Khosla, S. and Spelsberg, T.C. (2003) Estrogen receptor isoform-specific regulation of endogenous gene expression in human osteoblastic cell lines expressing either ERalpha or ERbeta. *J. Cell. Biochem.,* **90,** 315-326.

Nachtigall, L.E., Raju, U., Banerjee, S., Wan, L. and Levitz, M. (2000) Serum estradiol-binding profiles in postmenopausal women undergoing three common estrogen replacement therapies: associations with sex hormone-binding globulin, estradiol, and estrone levels. *Menopause,* **7,** 243-250.

Nelson, H.D., Humphrey, L.L., Nygren, P., Teutsch, S.M. and Allan, J.D. (2002) Postmenopausal hormone replacement therapy: scientific review. *JAMA,* **288,** 872-881.

NHI Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *J. Am. Med. Assoc*., **285**, 785-795.

North American Menopause, Society. (2004) Treatment of menopause-associated vasomotor symptoms: position statement of The North American Menopause Society. *Menopause,* **11,** 11-33.

Nugent, A.G., Leung, K.C., Sullivan, D., Reutens, A.T. and Ho, K.K. (2003) Modulation by progestogens of the effects of oestrogen on hepatic endocrine function in postmenopausal women. *Clin. Endocrinol,* **59,** 690-698.

Närhi, T.O., Ainamo, A. and Meurman, J.H. (1993) Salivary yeasts, saliva, and oral mucosa in the elderly. *J. Dent. Res.,* **72,** 1009-1014.

Närhi, T.O. (1994) Prevalence of subjective feelings of dry mouth in the elderly. *J. Dent. Res.,* **73,** 20-25.

Närhi, T.O., Meurman, J.H. and Ainamo, A. (1999) Xerostomia and hyposalivation: causes, consequences and treatment in the elderly. *Drugs Aging,* **15,** 103-116

O'Connell, M.B. (1999) Prevention and treatment of osteoporosis in the elderly. *Pharmacotherapy,* **19,** 7S-20S.

Odvina, C.V., Zerwekh, J.E., Rao, D.S., Maalouf, N., Gottschalk, F.A. and Pak, C.Y. (2005) Severely suppressed bone turnover: a potential complication of alendronate therapy. *J. Clin. Endocrinol. Metab.,* **90,** 1294-1301.

Oger, E., Alhenc-Gelas, M., Plu-Bureau, G., Mennen, L., Cambillau, M., Guize, L., Pujol, Y. and Scarabin, P. (2001) Association of circulating cellular adhesion molecules with menopausal status and hormone replacement therapy. Time-dependent change in transdermal, but not oral estrogen users. *Thromb. Res.,* **101,** 35-43.

Oldenhave, A., Jaszmann, L.J., Haspels, A.A. and Everaerd, W.T. (1993) Impact of climacteric on well-being. A survey based on 5213 women 39 to 60 years old. *Am. J. Obstet. Gynecol.,* **168,** 772-780.

Oldenhave, A. and Netelenbos, C. (1994) Pathogenesis of climacteric complaints: ready for the change? *Lancet,* **343,** 649-653.

Ooms, M.E., Roos, J.C., Bezemer, P.D., van der Vijgh, W.J., Bouter, L.M. and Lips, P. (1995) Prevention of bone loss by vitamin D supplementation in elderly women: a randomized double-blind trial. *J. Clin. Endocrinol. Metab.,* **80,** 1052-1058.

Owen, C.A., Hu, Z., Lopez-Otin, C. and Shapiro, S.D. (2004) Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J. Immunol.,* **172,** 7791-7803.

Paganini-Hill, A. (1995) The benefits of estrogen replacement therapy on oral health. The Leisure World cohort. *Arch. Intern. Med.,* **155,** 2325-2329.

Pajukoski, H., Meurman, J.H., Snellman-Gröhn, S., Keinänen, S. and Sulkava, R. (1997) Salivary flow and composition in elderly patients referred to an acute care geriatric ward. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.,* **84,** 265-271.

Pajukoski, H., Meurman, J.H., Halonen, P. and Sulkava, R. (2001) Prevalence of subjective dry mouth and burning mouth in hospitalized elderly patients and outpatients in relation to saliva, medication, and systemic diseases. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.,* **92,** 641-649.

Palomba, S., Orio, F.,Jr, Colao, A., di Carlo, C., Sena, T., Lombardi, G., Zullo, F. and Mastrantonio, P. (2002) Effect of estrogen replacement plus low-dose alendronate treatment on bone density in surgically postmenopausal women with osteoporosis. *J. Clin. Endocrinol. Metab.,* **87,** 1502-1508.

Papadimitropoulos, E., Wells, G., Shea, B., Gillespie, W., Weaver, B., Zytaruk, N., Cranney, A., Adachi, J., Tugwell, P., Josse, R., Greenwood, C. and Guyatt, G., Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. (2002) Meta-analyses of therapies for postmenopausal osteoporosis. VIII: Meta-analysis of the efficacy of vitamin D treatment in preventing osteoporosis in postmenopausal women. *Endocr. Rev.,* **23,** 560-569.

Parikka, V., Lehenkari, P., Sassi, M.L., Halleen, J., Risteli, J., Härkönen, P. and Väänänen, H.K. (2001) Estrogen reduces the depth of resorption pits by disturbing the organic bone matrix degradation activity of mature osteoclasts. *Endocrinology,* **142,** 5371-5378.

Payne, J.B., Reinhardt, R.A., Nummikoski, P.V. and Patil, K.D. (1999) Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. *Osteoporos. Int.,* **10,** 34-40.

Pirilä, E., Ramamurthy, N., Maisi, P., McClain, S., Kucine, A., Wahlgren, J., Golub, L., Salo, T. and Sorsa, T. (2001) Wound healing in ovariectomized rats: effects of chemically modified tetracycline (CMT-8) and estrogen on matrix metalloproteinases -8, -13 and type I collagen expression. *Curr. Med. Chem.,* **8,** 281-294.

Pols, H.A., Felsenberg, D., Hanley, D.A., Stepan, J., Munoz-Torres, M., Wilkin, T.J., Qin-sheng, G., Galich, A.M., Vandormael, K., Yates, A.J. and Stych, B. (1999) Multinational, placebo-controlled, randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: results of the FOSIT study. Foxamax International Trial Study Group. *Osteoporos. Int.,* **9,** 461-468.

Prelevic, G.M., Kwong, P., Byrne, D.J., Jagroop, I.A., Ginsburg, J. and Mikhailidis, D.P. (2002) A cross-sectional study of the effects of hormon replacement therapy on the cardiovascular disease risk profile in healthy postmenopausal women. *Fertil. Steril.,* **77,** 945-951.

Prestwood, K.M., Kenny, A.M., Kleppinger, A. and Kulldorff, M. (2003) Ultralow-dose micronized 17beta-estradiol and bone density and bone metabolism in older women: a randomized controlled trial. *JAMA,* **290,** 1042-1048.

Raisz, L.G. and Prestwood, K.M. (1998) Estrogen and the risk of fracture--new data, new questions. *N. Engl. J. Med.,* **339,** 767-768.

Rapuri, P.B., Gallagher, J.C. and Haynatzki, G. (2004) Endogenous levels of serum estradiol and sex hormone binding globulin determine bone mineral density, bone remodeling, the rate of bone loss, and response to treatment with estrogen in elderly women. *J. Clin. Endocrinol. Metab.,* **89,** 4954-4962.

Raudaskoski, T., Tomas, C. and Laatikainen, T. (1999) Insulin sensitivity during postmenopausal hormone replacement with transdermal estradiol and intrauterine levonorgestrel. *Acta Obstet. Gynecol. Scand.,* **78,** 540-545.

Raz, R. and Stamm, W.E. (1993) A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. *N. Engl. J. Med.,* **329,** 753-756.

Recker, R.R., Hinders, S., Davies, K.M., Heaney, R.P., Stegman, M.R., Lappe, J.M. and Kimmel, D.B. (1996) Correcting calcium nutritional deficiency prevents spine fractures in elderly women. *J. Bone Miner. Res.,* **11,** 1961-1966.

Recker, R.R., Davies, K.M., Dowd, R.M. and Heaney, R.P. (1999) The effect of low-dose continuous estrogen and progesterone therapy with calcium and vitamin D on bone in elderly women. A randomized, controlled trial. *Ann. Intern. Med.,* **130,** 897-904.

Reginster, J.Y., Seeman, E., De Vernejoul, M.C., Adami, S., Compston, J., Phenekos, C., Devogelaer, J.P., Curiel, M.D., Sawicki, A. and Goemaere, S., Sorensen,O.H., Felsenberg,D. and Meunier,P.J. (2005) Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J. Clin. Endocrinol. Metab.,* **90,** 2816-2822.

Reinhardt, R.A., Payne, J.B., Maze, C.A., Patil, K.D., Gallagher, S.J. and Mattson, J.S. (1999) Influence of estrogen and osteopenia/osteoporosis on clinical periodontitis in postmenopausal women. *J. Periodontol.,* **70,** 823-828.

Rich-Edwards, J.W., Manson, J.E., Hennekens, C.H. and Buring, J.E. (1995) The primary prevention of coronary heart disease in women. *N. Engl. J. Med.,* **332,** 1758-1766.

Ridker, P.M., Buring, J.E., Shih, J., Matias, M. and Hennekens, C.H. (1998) Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation,* **98,** 731-733.

Ridker, P.M., Hennekens, C.H., Rifai, N., Buring, J.E. and Manson, J.E. (1999) Hormone replacement therapy and increased plasma concentration of C-reactive protein. *Circulation,* **100,** 713-716.

Ridker, P.M., Hennekens, C.H., Buring, J.E. and Rifai, N. (2000) C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med.,* **342,** 836-843.

Riggs, B.L., Tsai, K.S. and Mann, K.G. (1986) Effect of acute increases in bone matrix degradation on circulating levels of bone-Gla protein. *J. Bone Miner. Res.,* **1,** 539-542.

Riggs, B.L., Khosla, S. and Melton, L.J.,3rd (2002) Sex steroids and the construction and conservation of the adult skeleton. *Endocr. Rev.,* **23,** 279-302.

Riman, T., Nilsson, S. and Persson, I.R. (2004) Review of epidemiological evidence for reproductive and hormonal factors in relation to the risk of epithelial ovarian malignancies. *Acta Obstet. Gynecol. Scand.,* **83,** 783-795.

Risteli, J., Elomaa, I., Niemi, S., Novamo, A. and Risteli, L. (1993) Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. *Clin. Chem.,* **39,** 635-640.

Rocha, M.L., Malacara, J.M., Sanchez-Marin, F.J., Vazquez de la Torre,C.J. and Fajardo, M.E. (2004) Effect of alendronate on periodontal disease in postmenopausal women: a randomized placebo-controlled trial. *J. Periodontol.,* **75,** 1579-1585.

Rodan, G.A., Seedor, J.G. and Balena, R. (1993) Preclinical pharmacology of alendronate. *Osteoporos. Int.,* **3,** S7-12.

Rogers, A., Saleh, G., Hannon, R.A., Greenfield, D. and Eastell, R. (2002) Circulating estradiol and osteoprotegerin as determinants of bone turnover and bone density in postmenopausal women. *J. Clin. Endocrinol. Metab.,* **87,** 4470-4475.

Ronderos, M., Jacobs, D.R., Himes, J.H. and Pihlstrom, B.L. (2000) Associations of periodontal disease with femoral bone mineral density and estrogen replacement therapy: cross-sectional evaluation of US adults from NHANES III. *J. Clin. Periodontol.,* **27,** 778-786.

Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C., Kotchen, J.M. and Ockene, J., WHI, Investigators. (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA,* **288,** 321-333.

Salmi T, Paldán M, Klaukka T. (2004) Vaihdevuosihormonien käyttö on vähentynyt maan kaikissa osissa. *Suom Lääkäril*., **59**,1042-1045.

Salo, T., Mäkelä, M., Kylmäniemi, M., Autio-Harmainen, H. and Larjava, H. (1994) Expression of matrix metalloproteinase-2 and -9 during early human wound healing. *Lab. Invest.,* **70,** 176-182.

Samsioe, G. (2002) The role of ERT/HRT. *Best Pract. Res. Clin. Obstet. Gynaecol.,* **16,** 371-381.

Sarkar S., Mitlak B.H., Wong M. Stock JL., Black DM. Harper KD. (2002) Relationships between bone mineral density and incident vertebral fracture risk with raloxifene therapy. *J.Bone Miner.Res*. **17**(1):1-10.

Sarkar, S., Reginster, J.Y., Crans, G.G., Diez-Perez, A., Pinette, K.V. and Delmas, P.D. (2004) Relationship between changes in biochemical markers of bone turnover and BMD to predict vertebral fracture risk. *J. Bone Miner. Res.,* **19,** 394-401.

Sarrel, P.M. (2002) Androgen deficiency: menopause and estrogen-related factors. *Fertil. Steril.,* **77,** S63-7.Sattar, N., Perera, M., Small, M. and Lumsden, M.A. (1999) Hormone replacement therapy and sensitive C-reactive protein concentrations in women with type-2 diabetes. *Lancet,* **354,** 487-488.

Scarabin, P.Y., Oger, E., Plu-Bureau, G. and EStrogen and THromboEmbolism Risk Study,Group (2003) Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet,* **362,** 428-432.

Schneider, D.L., Barrett-Connor, E.L. and Morton, D.J. (1997) Timing of postmenopausal estrogen for optimal bone mineral density. The Rancho Bernardo Study. *JAMA,* **277,** 543-547.

Seeman, E. (1997) Osteoporosis: trials and tribulations. *Am. J. Med.,* **103,** 74S-87S.

Seeman, E. (2004) Estrogen, androgen, and the pathogenesis of bone fragility in women and men. *Curr. osteoporos rep.,* **2,** 90-96.

Shea, B., Wells, G., Cranney, A., Zytaruk, N., Robinson, V., Griffith, L., Ortiz, Z., Peterson, J., Adachi, J., Tugwell, P. and Guyatt, G., Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. (2002) Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocr. Rev.,* **23,** 552-559.

Sintonen, H. (1994) Outcome Measurement in Acid-Related Diseases. *PharmacoEconomics,* **5,** 17-24.

Sintonen, H. (2001) The 15D instrument of health-related quality of life: properties and applications. *Ann. Med.,* **33,** 328-336.

Sintonen, H., Johansson, S., Ohinmaa, A., Apajasalo, M., Kainulainen, P. and Heikkinen, J. (2003) Measuring health-related quality of life in women on hormone replacement therapy. *Expert Rev Pharmacoeconomics Outcomes Res.,* **3,** 351-361.

Sit,A.S., Modugno,F., Weissfeld,J.L., Berga,S.L., Ness,R.B. (2002) Hormone replacement therapy formulations and risk of epithelial ovarian carcinoma. *Gynecol. Oncol*. **86**, 118-123.

Sites, C.K., Toth, M.J., Cushman, M., L´Hommedieu, G.D., Tchernof, A., Tracy, R.P. and Poehlman, E.T. (2002) Menopause-related differences in inflammation markers and their relationship to body fat distribution and insulin-stimulated glucose disposal. *Fertil. Steril.*, **77**,128-135.

Sorsa, T., Mäntylä, P., Ronka, H., Kallio, P., Kallis, G.B., Lundqvist, C., Kinane, D.F., Salo, T., Golub, L.M., Teronen, O. and Tikanoja, S. (1999) Scientific basis of a matrix metalloproteinase-8 specific chair-side test for monitoring periodontal and peri-implant health and disease. *Ann. N. Y. Acad. Sci.,* **878,** 130-140.

Sorsa, T., Tjäderhane, L. and Salo, T. (2004) Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis.,* **10,** 311-318.

Sourander,L., Rajala,T., Räihä,I., Mäkinen,J., Erkkola,R., Helenius,H. (1998) Cardiovascular and cancer morbidity and mortality and sudden cardiac death in postmenopausal women on oestrogen replacement therapy (ERT). *Lancet,* **352,**1965-1969.

Speroff, L., Rowan, J., Symons, J., Genant, H. and Wilborn, W. (1996) The comparative effect on bone density, endometrium, and lipids of continuous hormones as replacement therapy (CHART study). A randomized controlled trial. *JAMA,* **276,** 1397-1403.

Stavem, K. (1999) Reliability, validity and responsiveness of two multiattribute utility measures in patients with chronic obstructive pulmonary disease. *Qual. Life Res.,* **8,** 45-54.

Stearns, V., Ullmer, L., Lopez, J.F., Smith, Y., Isaacs, C. and Hayes, D. (2002) Hot flushes. *Lancet,* **360,** 1851-1861.

Stefanick, M.L., Anderson, G.L., Margolis, K.L., Hendrix, S.L., Rodabough, R.J., Paskett, E.D., Lane, D.S., Hubbell, F.A., Assaf, A.R., Sarto, G.E., Schenken,R.S., Yasmeen, S., Lessin, L. and Chlebowski, R.T., for the WHI Investigators (2006) Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. *JAMA,* **295,** 1647-1657.

Stein, G.S., Lian, J.B., Stein, J.L., Van Wijnen, A.J. and Montecino, M. (1996) Transcriptional control of osteoblast growth and differentiation. *Physiol. Rev.,* **76,** 593-629.

Stevenson M, Lloyd Jones M, De Nigris E, Brewer N, Davis S, Oakley J. (2005) A systematic review and economic evaluation of alendronate, etidronate, risedronate, raloxifene and teriparatide for the prevention and treatment of postmenopausal osteoporosis. *Health Technol Assess.,* **9**,1-160.

Tähtelä, R. and Tholix, E. (1996) Serum concentrations of type I collagen carboxyterminal telopeptide (ICTP) and type I procollagen carboxy-and aminoterminal propeptides (PICP, PINP) as markers of metastatic bone disease in breast cancer. *Anticancer Res.,* **16,** 2289-2293.

Tanko LB, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR. (2005) Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res*., **20**,1912-1920.

Tarkkila, L., Linna, M., Tiitinen, A., Lindqvist, C. and Meurman, J.H. (2001) Oral symptoms at menopause--the role of hormone replacement therapy. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.,* **92,** 276-280.

Tenenbaum, H.C., Shelemay, A., Girard, B., Zohar, R. and Fritz, P.C. (2002 Jul) Bisphosphonates and periodontics: potential applications for regulation of bone mass in the periodontium and other therapeutic/diagnostic uses. *J. Periodontol.,* **73,** 813-822.

Teronen, O., Heikkilä, P., Konttinen, Y.T., Laitinen, M., Salo, T., Hanemaaijer, R., Teronen, A., Maisi, P. and Sorsa, T. (1999) MMP inhibition and downregulation by bisphosphonates. *Ann. N. Y. Acad. Sci.,* **878,** 453-465.

Tezal, M., Wactawski-Wende, J., Grossi, S.G., Ho, A.W., Dunford, R. and Genco, R.J. (2000) The relationship between bone mineral density and periodontitis in postmenopausal women. *J. Periodontol.,* **71,** 1492-1498.

The Women's Health Initiative Steering,Committee (2004) Effects of Conjugated Equine Estrogen in Postmenopausal Women With Hysterectomy: The Women's Health Initiative Randomized Controlled Trial. *JAMA,* **291,** 1701-1712.

The Writing Group for the PEPI,trial (1995) Effects of Estrogen or Estrogen/Progestin Regimens on Heart Disease Risk Factors in Postmenopausal Women: The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA,* **273,** 199-208.

Thiebaud, D., Sauty, A., Burckhardt, P., Leuenberger, P., Sitzler, L., Green, J.R., Kandra, A., Zieschang, J. and Ibarra de Palacios, P. (1997) An in vitro and in vivo study of cytokines in the acute-phase response associated with bisphosphonates. *Calcif. Tissue Int.,* **61,** 386-392.

Tintut,Y. and Demer, L.L. (2001) Recent advances in multifactorial regulation of vascular calcification. Current Opinion in Lipidology. **12**(5), 555-560.

Tiras, M.B., Noyan, V., Yildiz, A., Yildirim, M. and Daya, S. (2000) Effects of alendronate and hormone replacement therapy, alone or in combination, on bone mass in postmenopausal women with osteoporosis: a prospective, randomized study. *Hum. Reprod.,* **15,** 2087-2092.

Tonino, R.P., Meunier, P.J., Emkey, R., Rodriguez-Portales, J.A., Menkes, C.J., Wasnich, R.D., Bone, H.G., Santora, A.C., Wu, M., Desai, R. and Ross, P.D. (2000) Skeletal benefits of alendronate: 7-year treatment of postmenopausal osteoporotic women. Phase III Osteoporosis Treatment Study Group. *J. Clin. Endocrinol. Metab.,* **85,** 3109-3115.

Topo, P., Luoto, R., Hemminki, E. and Uutela, A. (1999) Declining socioeconomic differences in the use of menopausal and postmenopausal hormone therapy in Finland. *Maturitas,* **32,** 141-145.

Torgerson, D.J. and Bell-Syer, S.E. (2001) Hormone replacement therapy and prevention of nonvertebral fractures: a meta-analysis of randomized trials. *JAMA,* **285,** 2891-2897.

Tremollieres, F.A., Cigagna, F., Alquier, C., Cauneille, C., Pouilles, J. and Ribot, C. (2000) Effect of hormone replacement therapy on age-related increase in carotid artery intima-media thickness in postmenopausal women. *Atherosclerosis*, **153**, 81-88.

van Baal, W.M., Kenemans, P., van der Mooren, M.J., Kessel, H., Emeis, J.J. and Stehouwer, C.D. (1999) Increased C-reactive protein levels during short-term hormone replacement therapy in healthy postmenopausal women. *Thromb. Haemost.,* **81,** 925-928.

Vane, J.R., Anggard, E.E. and Botting, R.M. (1990) Regulatory functions of the vascular endothelium. *N. Engl. J. Med.,* **323,** 27-36.

Vehkavaara, S., Silveira, A., Hakala-Ala-Pietilä, T., Virkamäki, A., Hovatta, O., Hamsten, A., Taskinen, M.R. and Yki-Järvinen, H. (2001) Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. *Thromb. Haemost.,* **85,** 619-625.

Viscoli, C.M., Brass, L.M., Kernan, W.N., Sarrel, P.M., Suissa, S. and Horwitz, R.I. (2001) A clinical trial of estrogen-replacement therapy after ischemic stroke.[see comment]. *N. Engl. J. Med.,* **345,** 1243-1249.

Wactawski-Wende, J., Grossi, S.G., Trevisan, M., Genco, R.J., Tezal, M., Dunford, R.G., Ho, A.W., Hausmann, E. and Hreshchyshyn, M.M. (1996) The role of osteopenia in oral bone loss and periodontal disease. *J. Periodontol.,* **67,** 1076-1084.

Wactawski-Wende, J. (2001) Periodontal diseases and osteoporosis: association and mechanisms. *Ann. Periodontol.,* **6,** 197-208.

Wactawski-Wende, J., Hausmann, E., Hovey, K., Trevisan, M., Grossi, S. and Genco, R.J. (2005) The association between osteoporosis and alveolar crestal height in postmenopausal women. *J. Periodontol.,* **76,** 2116-2124.

Wahner, H.W. (1989) Measurements of bone mass and bone density. *Endocrinol. Metab. Clin. North Am.,* **18,** 995-1012.

Wajchenberg, B.L. (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr. Rev.,* **21,** 697-738.

Wardrop, R.W., Hailes, J., Burger, H. and Reade, P.C. (1989) Oral discomfort at menopause. *Oral Surg. Oral Med. Oral Pathol.,* **67,** 535-540.

Weiss, S.R., Ellman, H. and Dolker, M. (1999) A randomized controlled trial of four doses of transdermal estradiol for preventing postmenopausal bone loss. Transdermal Estradiol Investigator Group. *Obstet. Gynecol.,* **94,** 330-336.

Wells, G., Tugwell, P., Shea, B., Guyatt, G., Peterson, J., Zytaruk, N., Robinson, V., Henry, D., O'Connell, D. and Cranney, A., the osteoporosis methodology group, and the osteoporosis research advisory group. (2002) Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. *Endocr. Rev.,* **23,** 529-539.

Westhoff, C., Heller, D., Drosinos, S. and Tancer, L. (2000) Risk factors for hyperplasia-associated versus atrophy-associated endometrial carcinoma. *Am. J. Obstet. Gynecol.,* **182,** 506-508.

Wiklund, I., Berg, G., Hammar, M., Karlberg, J., Lindgren, R. and Sandin, K. (1992) Long-term effect of transdermal hormonal therapy on aspects of quality of life in postmenopausal women. *Maturitas,* **14,** 225-236.

Wilcox, J.G., Hatch, I.E., Gentzschein, E., Stanczyk, F.Z. and Lobo, R.A. (1997) Endothelin levels decrease after oral and nonoral estrogen in postmenopausal women with increased cardiovascular risk factors. *Fertil. Steril.,* **67,** 273-277.

Wimalawansa, S.J. (1995) Combined therapy with estrogen and etidronate has an additive effect on bone mineral density in the hip and vertebrae: four-year randomized study. *Am. J. Med.,* **99,** 36-42.

Wimalawansa, S.J. (1998) A four-year randomized controlled trial of hormone replacement and bisphosphonate, alone or in combination, in women with postmenopausal osteoporosis. *Am. J. Med.,* **104,** 219-226.

Yalcin, F., Gurgan, S. and Gurgan, T. (2005) The effect of menopause, hormone replacement therapy (HRT), alendronate (ALN), and calcium supplements on saliva. *J. Contemp. Dent. Pract.,* **6,** 10-17.

Ylikangas S, Sintonen H, Heikkinen J. (2005) Decade-long use of continuous combined hormone replacement therapy is associated with better health-related quality of life in postmenopausal women, as measured by the generic 15D instrument. J Br Menopause Soc., **11**(4): 145-51.

Ylikorkala, O., Pekonen, F. and Viinikka, L. (1986) Renal prostacyclin and thromboxane in normotensive and preeclamptic pregnant women and their infants. *J. Clin. Endocrinol. Metab.,* **63,** 1307-1312.

Ylikorkala, O., Orpana, A., Puolakka, J., Pyörälä, T. and Viinikka, L. (1995) Postmenopausal hormonal replacement decreases plasma levels of endothelin-1. *J. Clin. Endocrinol. Metab.,* **80,** 3384-3387.

Ylitalo, R., Kalliovalkama, J., Wu, X., Kankaanranta, H., Salenius, J.P., Sisto, T., Lähteenmäki, T., Ylitalo, P. and Porsti, I. (1998) Accumulation of bisphosphonates in human artery and their effects on human and rat arterial function in vitro. *Pharmacol. Toxicol.,* **83,** 125-131.

Ylitalo, R. (2000) Bisphosphonates and atherosclerosis. *Gen. Pharmacol.,* **35,** 287-296.

Zanger, D., Yang, B.K., Ardans, J., Waclawiw, M.A., Csako, G., Wahl, L.M. and Cannon, R.O.,3rd (2000) Divergent effects of hormone therapy on serum markers of inflammation in postmenopausal women with coronary artery disease on appropriate medical management. *J. Am. Coll. Cardiol.,* **36,** 1797-1802.

Zethraeus, N., Johannesson, M., Henriksson, P. and Strand, R.T. (1997) The impact of hormone replacement therapy on quality of life and willingness to pay. *Br. J. Obstet. Gynaecol.,* **104,** 1191-1195.

Zichella, L. (1993) Clinical management of the menopausal woman. *Int. J. Fertil. Menopausal Stud.,* **38,** 15-22.

Appendices:
QUALITY OF LIFE QUESTIONNAIRE (15D©)

Please read through all the alternative responses to each question before placing a cross (x) against the alternative which best describes **your present health status**. Continue through all 15 questions in this manner, giving only **one** answer to each.

QUESTION 1. MOBILITY
1 ( ) I am able to walk normally (without difficulty) indoors, outdoors and on stairs.
2 ( ) I am able to walk without difficulty indoors, but outdoors and/or on stairs I have slight difficulties.
3 ( ) I am able to walk without help indoors (with or without an appliance), but outdoors and/or on stairs only with considerable difficulty or with help from others.
4 ( ) I am able to walk indoors only with help from others.
5 ( ) I am completely bed-ridden and unable to move about.

QUESTION 2. VISION
1 ( ) I see normally, i.e. I can read newspapers and TV text without difficulty (with or without glasses).
2 ( ) I can read papers and/or TV text with slight difficulty (with or without glasses).
3 ( ) I can read papers and/or TV text with considerable difficulty (with or without glasses).
4 ( ) I cannot read papers or TV text either with glasses or without, but I can see enough to walk about without guidance.
5 ( ) I cannot see enough to walk about without a guide, i.e. I am almost or completely blind.

QUESTION 3. HEARING
1 ( ) I can hear normally, i.e. normal speech (with or without a hearing aid).
2 ( ) I hear normal speech with a little difficulty.
3 ( ) I hear normal speech with considerable difficulty; in conversation I need voices to be louder than normal.
4 ( ) I hear even loud voices poorly; I am almost deaf.
5 ( ) I am completely deaf.

QUESTION 4. BREATHING
1 ( ) I am able to breathe normally, i.e. with no shortness of breath or other breathing difficulty.
2 ( ) I have shortness of breath during heavy work or sports, or when walking briskly on flat ground or slightly uphill.
3 ( ) I have shortness of breath when walking on flat ground at the same speed as others my age.
4 ( ) I get shortness of breath even after light activity, e.g. washing or dressing myself.
5 ( ) I have breathing difficulties almost all the time, even when resting.

QUESTION 5. SLEEPING
1 ( ) I am able to sleep normally, i.e. I have no problems with sleeping.
2 ( ) I have slight problems with sleeping, e.g. difficulty in falling asleep, or sometimes waking at night.
3 ( ) I have moderate problems with sleeping, e.g. disturbed sleep, or feeling I have not slept enough.
4 ( ) I have great problems with sleeping, e.g. having to use sleeping pills often or routinely, or usually waking at night and/or too early in the morning.
5 ( ) I suffer severe sleeplessness, e.g. sleep is almost impossible even with full use of sleeping pills, or staying awake most of the night.

QUESTION 6. EATING
1 ( ) I am able to eat normally, i.e. with no help from others.
2 ( ) I am able to eat by myself with minor difficulty (e.g. slowly, clumsily, shakily, or with special appliances).
3 ( ) I need some help from another person in eating.
4 ( ) I am unable to eat by myself at all, so I must be fed by another person.
5 ( ) I am unable to eat at all, so I am fed either by tube or intravenously.

QUESTION 7. SPEECH
1 ( ) I am able to speak normally, i.e. clearly, audibly and fluently.
2 ( ) I have slight speech difficulties, e.g. occasional fumbling for words, mumbling, or changes of pitch.
3 ( ) I can make myself understood, but my speech is e.g. disjointed, faltering, stuttering or stammering.
4 ( ) Most people have great difficulty understanding my speech.
5 ( ) I can only make myself understood by gestures.

QUESTION 8. ELIMINATION
1 ( ) My bladder and bowel work normally and without problems.
2 ( ) I have slight problems with my bladder and/or bowel function, e.g. difficulties with urination, or loose or hard bowels.
3 ( ) I have marked problems with my bladder and/or bowel function, e.g. occasional 'accidents', or severe constipation or diarrhea.

4 ( ) I have serious problems with my bladder and/or bowel function, e.g. routine 'accidents', or need of catheterization or enemas.
5 ( ) I have no control over my bladder and/or bowel function.

QUESTION 9. USUAL ACTIVITIES

1 ( ) I am able to perform my usual activities (e.g. employment, studying, housework, free-time activities) without difficulty.
2 ( ) I am able to perform my usual activities slightly less effectively or with minor difficulty.
3 ( ) I am able to perform my usual activities much less effectively, with considerable difficulty, or not completely.
4 ( ) I can only manage a small proportion of my previously usual activities.
5 ( ) I am unable to manage any of my previously usual activities.

QUESTION 10. MENTAL FUNCTION

1 ( ) I am able to think clearly and logically, and my memory functions well
2 ( ) I have slight difficulties in thinking clearly and logically, or my memory sometimes fails me.
3 ( ) I have marked difficulties in thinking clearly and logically, or my memory is somewhat impaired.
4 ( ) I have great difficulties in thinking clearly and logically, or my memory is seriously impaired.
5 ( ) I am permanently confused and disoriented in place and time.

QUESTION 11. DISCOMFORT AND SYMPTOMS

1 ( ) I have no physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
2 ( ) I have mild physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
3 ( ) I have marked physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
4 ( ) I have severe physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.
5 ( ) I have unbearable physical discomfort or symptoms, e.g. pain, ache, nausea, itching etc.

QUESTION 12. DEPRESSION

1 ( ) I do not feel at all sad, melancholic or depressed.
2 ( ) I feel slightly sad, melancholic or depressed.
3 ( ) I feel moderately sad, melancholic or depressed.
4 ( ) I feel very sad, melancholic or depressed.
5 ( ) I feel extremely sad, melancholic or depressed.

QUESTION 13. DISTRESS

1 ( ) I do not feel at all anxious, stressed or nervous.
2 ( ) I feel slightly anxious, stressed or nervous.
3 ( ) I feel moderately anxious, stressed or nervous.
4 ( ) I feel very anxious, stressed or nervous.
5 ( ) I feel extremely anxious, stressed or nervous.

QUESTION 14. VITALITY

1 ( ) I feel healthy and energetic.
2 ( ) I feel slightly weary, tired or feeble.
3 ( ) I feel moderately weary, tired or feeble.
4 ( ) I feel very weary, tired or feeble, almost exhausted.
5 ( ) I feel extremely weary, tired or feeble, totally exhausted.

QUESTION 15. SEXUAL ACTIVITY

1 ( ) My state of health has no adverse effect on my sexual activity.
2 ( ) My state of health has a slight effect on my sexual activity.
3 ( ) My state of health has a considerable effect on my sexual activity.
4 ( ) My state of health makes sexual activity almost impossible.
5 ( ) My state of health makes sexual activity impossible.